Université de Sherbrooke

Rôle du facteur de transcription Hnf4alpha dans la réponse de la cellule épithéliale intestinale face aux infections bactériennes

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Mémoire présenté à la Faculté de médecine et des sciences de la santé
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Je dédie ce travail à mes grands-parents Papi et José, ma famille et surtout mes parents pour tout le soutien et l'amour inconditionnel

RESUME

Rôle du facteur de transcription Hnf4alpha dans la réponse de la cellule épithéliale intestinale face aux infections bactériennes

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Les maladies inflammatoires de l'intestin (MIIs) sont un groupe de troubles chroniques qui touchent plus de 270 000 personnes au Canada. De plus, les MIIs sont des maladies multifactorielles dépendant de dérèglements génétiques, immunitaires et environnementaux. L'épithélium gastro-intestinal joue un rôle important en tant que barrière. De plus, il est bien reconnu qu'un défaut d'intégrité de la barrière et de ses fonctions peuvent être impliqués dans le développement de ces maladies. D'autre part, notre laboratoire a montré que la délétion conditionnelle du récepteur nucléaire HNF4α dans l'épithélium intestinal de souris peut conduire au développement d'une inflammation chronique intestinale. Cependant, l'impact de la perte de ce facteur transcriptionnel sur la barrière épithéliale est encore controversé. Dans ce mémoire, nous avons tenté d'évaluer l'impact de la délétion de HNF4α sur la barrière épithéliale lors d'infections bactériennes. La génération de mutantes HNF4α dans l'épithélium intestinal a été réalisée en croisant des animaux *Hnf4a*$^{loxP/loxP}$ C57BL/6 avec un modèle murin *Villin*-CreERT2, dans lequel la délétion inductible du gène a été conditionnée par l'administration de tamoxifène chez la souri à un jeune âge adulte. Chez les souris *Hnf4a*$^{\Delta IEC\text{-}ind}$, une augmentation du passage du FITC-dextran dans la circulation sanguine a révélé que l'épithélium intestinal présentait une augmentation de la perméabilité associée à la mutation. Il est intéressant de noter que l'infection par voie orale avec une souche de *Salmonella* Typhimurium déficiente pour un gène impliqué dans l'invasion n'a pas montré de différences significatives en termes de charge bactérienne détectée pour *Hnf4a*$^{\Delta IEC\text{-}ind}$ et les souris témoins. Puis, l'expression génique des cibles sélectionnées a été évaluée chez le modèle *Hnf4a*$^{\Delta IEC\text{-}ind}$ par qPCR. En ce sens, la perte de HNF4α semble affecter l'expression de différentes molécules des jonctions apicales, liées à la fois à la perméabilité paracellulaire (*Cldn2*) et au transport ionique (*Cldn15*). De même, nous prouvons que les souris *Hnf4a*$^{\Delta IEC\text{-}ind}$ montrent une expression accrue de gènes liés à la protection et à l'intégrité de la barrière épithéliale, tels que *Retlnb*, *Muc2*, ainsi que les peptides antimicrobiens *Defa5* et *Defa20*, une observation plus marquée chez les animaux mâles. Les examens histologiques chez les souris *Hnf4a*$^{\Delta IEC\text{-}ind}$ ont démontré des augmentations du nombre et de la taille des cellules caliciformes, principalement au niveau de la crypte iléale. En plus, la délétion de *Hnf4a* a non seulement modifié le patron d'expression de la fucosylation dans l'iléon après l'infection, mais aussi, a semblé influencer l'expression des gènes associés à la spécification et à la différenciation des cellules sécrétoires. Globalement, nos résultats suggèrent que HNF4α pourrait jouer un rôle adaptatif important en tant que médiateur de la fonction de barrière épithéliale intestinale en présence d'infections bactériennes.

Mots clés: HNF4α, épithélium intestinal, barrière, tamoxifène, infection, maladies inflammatoires de l'intestin (MIIs)

SUMMARY

Role of the transcription factor Hnf4alpha in intestinal epithelial cell response to bacterial infections

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Master's thesis presented to the Faculty of Medicine and Health Sciencies in view of obtaining a Master of Science (MSc.) in Cell Biology. Université de Sherbrooke, Sherbrooke, Québec, Canada, J1H 5N4

Inflammatory bowel diseases (IBD) are a group of chronic disorders that affect more than 270,000 individuals in Canada. In addition, IBD are multifactorial diseases depending on genetic, immune and environmental dysregulations. The gastrointestinal epithelium plays an important role as a barrier. Also, it is well recognized that a defect in the integrity of the barrier and its functions may be involved in the development of these diseases. On the other hand, our laboratory has shown that the conditional deletion of HNF4alpha nuclear receptor (HNF4α) in the intestinal epithelium of mice can lead to the development of intestinal chronic inflammation. However, the impact of the loss of this transcriptional factor on the epithelial barrier is still controversial. The aim of this thesis was to evaluate the impact of *Hnf4a* deletion on the epithelial barrier during bacterial infections. The HNF4α mutants generation in the intestinal epithelium was achieved by crossing *Hnf4a*$^{loxP/loxP}$ C57BL/6 animals with a Villin-CreERT2 mouse model, in which the inducible gene deletion was caused by the administration of tamoxifen in mice at young adult age. In *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice, an increase in the passage of FITC-dextran into the blood circulation revealed that the intestinal epithelium presented an increase in permeability associated with the mutation. Interestingly, oral infection with an invasion-deficient *Salmonella* Typhimurium strain did not show relevant differences in terms of the bacterial load detected for *Hnf4a*$^{\Delta IEC\text{-}ind}$ and control littermate mice. Also, gene expression of selected targets was assessed in *Hnf4a*$^{\Delta IEC\text{-}ind}$ by qPCR. In this sense, the loss of HNF4α seemed to affect the expression of different molecules of the apical junctions, related to both paracellular permeability (*Cldn2*) and ion transport (*Cldn15*). Likewise, *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice showed increased expression of genes related to the protection and integrity of the epithelial barrier, such as *Retlnb*, *Muc2*, as well as the anti-microbial peptides *Defa5* and *Defa20*, an observation being more marked in male animals. Histological examinations showed that *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice display increases in the number and size of goblets cells, mainly at the level of the ileal crypt. Furthermore, *Hnf4a* deletion not only modified the expression patterns of fucosylation in the ileum after infection, but also, seemed to influence the expression of genes associated with the specification and differentiation of secretory cells. Globally, these results suggest that HNF4α could play an important adaptive role as a mediator of the intestinal epithelial barrier function in the presence of bacterial infections.

Keywords: HNF4α, intestinal epithelium, barrier, tamoxifen, infection, inflammatory bowel diseases (IBD)

TABLE OF CONTENTS

RESUME ... iii

SUMMARY ... iv

TABLE OF CONTENTS ... v

LIST OF FIGURES ... vii

LIST OF TABLES ... viii

LIST OF ABBREVIATIONS ... ix

1. INTRODUCTION ... 1

1.1 Gastrointestinal tract ... 1

1.2 Intestinal barrier ... 2

1.2.1 Goblet cells and mucus ... 3

1.2.2 The junctional complexes as a physical barrier of the intestinal epithelium ... 9

1.3 Inflammatory Bowel Diseases ... 12

1.3.1 Intestinal barrier dysfunction in IBD ... 12

1.3.2 Microbiota in IBD ... 15

1.3.3 Genetic variants and IBD ... 19

1.4 *HNF4A* as a susceptibility gene in IBD and its controversial role in the control of the intestinal epithelial barrier. ... 21

1.5 Premises and hypothesis ... 23

2. MATERIALS AND METHODS ... 24

2.1 Animal model: the conditional tamoxifen-inducible *Hnf4a* knockout mouse model ... 24

2.2 Mouse genotyping ... 24

2.3 *In vivo* intestinal permeability assessment ... 25

2.4 Mouse sacrifice and tissue fixation methods ... 25

2.5 Staining ... 25

2.5.1 Hematoxylin & Eosin (H&E) ... 25

2.5.2 Alcian Blue – Periodic acid Schiff (AB-PAS) ... 26

2.6 *Salmonella* Typhimurium infection ... 27

2.7 RNA isolation ... 27

2.8 Reverse transcription ... 28

2.9 Quantitative real-time RT-qPCR ... 28

2.10 Immunofluorescence ... 30

2.11 Fluorescence in situ hybridization (FISH) 31
2.12 Electron microscopy analysis 32
2.13 Extraction and quantification of proteins from animal tissue 32
2.14 IgA detection in the intestinal epithelium 33
2.15 Statistical analyses 33
3. RESULTS 34
3.1 Increased paracellular permeability of the intestinal mucosa in the absence of epithelial HNF4α. 34
3.2 Loss of HNF4α does not influence infection outcomes from attenuated *Salmonella* Typhimurium strain SB103 36
3.3 The apical junction complex appears to be modified in HNF4α$^{\Delta IEC\text{-}ind}$ mice 39
***3.4* HNF4α intestinal epithelial deletion alters the expression of different gene transcripts related to the protection and integrity of the epithelial barrier after *Salmonella* infection 40**
3.4.1 Apical complex junctions 40
3.4.2 Cytokines and immune system modulators 42
3.4.3 Antimicrobial peptides 44
3.4.4 Intestinal sIgA production 45
3.4.5 Mucus layer 47
***3.5 Hnf4a$^{\Delta IEC\text{-}ind}$* mice display increases in the small intestinal number and size of goblet cells after attenuated *Salmonella* infection. 48**
3.6 Deletion of HNF4α in the ileum of male mice impact gene transcript levels associated with secretory cells specification and differentiation 51
3.7 Loss of HNF4α seems to influence fucosylation patterns of infected female mice in the small intestine. 53
4. DISCUSSION 55
5. CONCLUSIONS 70
6. PERSPECTIVES 71
7. REMERCIEMENTS 73
8. REFERENCES 74
9. ANNEXES 105

LIST OF FIGURES

Figure 1. Composition of the intestinal epithelium……3
Figure 2. Structure of intestinal goblets cells……6
Figure 3. Distribution of the intercellular junctional complex among intestinal epithelial cells……...10
Figure 4. Different pathways and genes identified as risk factors in IBD pathology……...20
Figure 5. Intestinal permeability is increased in the absence of HNF4α……..35
Figure 6. Loss of HNF4α does not lead to increased susceptibility to *Salmonella* infection……37
Figure 7. Distension of the apical complex junctions in the absence of HNF4α……39
Figure 8. Expression of cell junction-associated components in the intestine of mice deleted for *Hnf4a*……...41
Figure 9. Expression of immune response modulators from the intestinal epithelium of control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ after infection with the attenuated *Salmonella* Typhimurium strain SB103……...43
Figure 10. Profile of AMP genes expression in the ileum of *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice during bacterial infection……..45
Figure 11. Quantification of total sIgA production in the intestine of mice after enteric infection……46
Figure 12. Altered gene expression for intestinal epithelial goblet cells of *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice after infection with invasive deficient *Salmonella* Typhimurium……..48
Figure 13. Histological and goblet cells analyses in the ileum of *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice after *Salmonella* Typhimurium strain SB103 infection……..49
Figure 14. Histological and goblet cells analyses in the colon of *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice after *Salmonella* Typhimurium strain SB103 infection……..50
Figure 15. Altered number and size of goblets cells in the ileum of infected *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice……...51
Figure 16. Loss of HNF4α enhance the expression of genes associated with goblet cells differentiation in the ileum of *Hnf4a*$^{\Delta IEC\text{-}ind}$ infected male mice……...52
Figure 17. Loss of HNF4α promotes abnormal fucosylation in infected mice…….54
Annexes
Figure 1. *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice have a thick internal mucus layer……..112
Figure 2. Epithelial 2D-Monolayers established from intestinal organoids derived from *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice……....113

LIST OF TABLES

Table 1. Primers used for PCR genotyping……..24
Table 2. Tissue rehydration and dehydration protocol…….....26
Table 3. Real-Time RT-qPCR primers (Mus musculus) …….....29
Table 4. Primary and secondary antibodies used for IF……31
Table 5. Fish probes primer sequences…….....32
Annexes
Table 1. (Supplemental materials and methods) Composition of the ad-FD^{+++} medium...110
Table 2. (Supplemental materials and methods) Composition of the WENR medium…..110
Table 3. (Supplemental results) Statistical analysis of the CFU obtained in mutant and control mice feces for each day of infection……...112

LIST OF ABBREVIATIONS

AIEC: adherent and invasive *Escherichia coli*
AJ: adherens juntions
Ang4: angiogenin 4
ATOH1/MATH1: atonal homolog 1
AMP: antimicrobial peptides
BSA: Bovine serum albumin
Casp 1: inflammatory caspase 1
CD: Crohn's disease
CDH1*:* E-cadherin
CLDN: Claudin human gene or protein
Cldn : Claudin mouse gene
cDNA: Complementary deoxyribonucleic acid
CDX2: caudal-type homeobox 2
CFU: colony forming units
DAPI: 4',6-diamidino-2-phenylindole
DNA: Deoxyribonucleic acid
dNTPs: Nucleoside triphosphates
Defa: α-Defensin
EDTA: Ethylenediaminetetraacetic acid
ER: Endoplasmatic Reticulum
Erα/β: Estrogen receptor alpha or beta
FCGBP: Fc fragment of IgG binding protein
FISH: Fluorescence *in situ* hybridization
FITC-dextran: polymer conjugated to Fluorescein isothiocyanate
FXR: Farnesoid X receptor
Fut2: fucosyltransferase 2
GATA4: GATA-binding family 4
GI: Gastrointestinal tract
GFI1: Growth factor independent 1
GR: Glucocorticoid receptor
GsdmD: Gasdermin D
GWAS: Genome-wide association studies
HES1: Hairy and enhancer-of-split 1
HNF4α: Hepatocyte nuclear factor 4 alpha (protein)
HNF4A: human gene
$Hnf4\alpha^{\Delta IEC\text{-}ind}$: Inducible deletion of HNF4α from the intestinal epithelium of mice
IBD: Inflammatory Bowel Disease
IEC: intestinal epithelial cells
IESC: intestinal epithelial stem cells
IL: Interleukin
JAM: junctional adhesion molecules
KLF4: Kruppel-like factor 4
Lyz: Lysozime

LoxP: Locus of X-ing over PI
LPS: Lipopolysaccharides
M: microfold cells
MAPK: Mitogen-activated protein kinases
MLCK: myosin light chain kinase
mRNA: Messenger ribonucleic acid
Muc: mucin mouse gene or protein
MUC: mucin human gene or protein
NAF: Sodium fluoride
NF-kB: Nuclear factor-kappa B
NHE3: transporter Na^+/ H^+ exchanger isoform 3
NR: nuclear receptor
Ocldn: occludin gene
PBS: Phosphate-buffered saline
PCR: Polymerization reaction
PFA: Paraformaldehyde
$sPLA_2$: phospholipase A2
pi: post-infection
PI3K: Phosphoinositide 3-kinase
pNPP: p-nitrophenyl-phosphate
PPARγ: Peroxisome Proliferator-Activated receptors
PRRs: Pattern recognition receptors
PSA: polysaccharide A
PXR: Pregnane x receptor
RELMβ: Resistin-like molecule β (gene *Reltnb*)
REGIII: Regenerating islet-derived protein III
RNA: Ribonucleic acid
RT: Room temperature
RT-qPCR: Reverse transcription polymerase chain reaction
sIgA: secretory immunoglobulin A
SCFA: short chain fatty acids
SGLT: Sodium-glucose co-transporter
SPDEF: SAM pointed domain containing ETS transcription factor
TAM: tamoxifen
TBP: TATA binding protein
TER: transepithelial electrical resistance
TF: transcriptional factor
TFF3: Trefoil factor 3
TJ: tight juntions
TLR: Toll-like receptors
TNFα: Tumor necrosis factor alpha
UC: Ulcerative colitis
UEA-1: *Ulex europaeus* agglutinin 1
VDR: Vitamin D receptors
ZO: zonula occluden

1. INTRODUCTION

1.1 Gastrointestinal tract

The gastrointestinal tract (GI) constitutes the main organ related to digestive functions including absorption of nutrients, water and electrolytes transport, and secretion of proteins to the intestinal lumen in order to maintain the correct balance or homeostasis. Subsequent to the stomach (Kong et al., 2018), the small intestine includes the duodenum, jejunum, and ileum. The following large intestine is separated into the proximal colon, transverse colon, distal colon, and the rectum. The colonic content is made up of a mixture of bile, secreted mucus, and molecules that cannot be digested or absorbed. Also, the colon contains the highest proportion of the commensal microbiota that colonizes the intestine, thus directly influencing the development and maturation of the immune system functions. Taking into account the latter, it is known that the intestinal epithelium also represents an effective barrier against the invasion of microorganisms or infectious agents that can cause various diseases. Besides, it hinders the passage of molecules with potentially harmful functions in the body (Turner, 2009).

The wall of the intestinal epithelium is made up of four layers including the mucosa, submucosa, muscularis and serosa. Within these layers, the mucosa is divided into the muscularis-mucosa (composed of several thin layers of smooth muscle fibers located underlying the lamina propria and related to the ability of the mucosa to fold and move), the lamina propria (found below the epithelium that houses a large number of immune cells and connective tissue) and finally the epithelium. In the small intestine, the epithelial surface forms finger-like protrusions which are called villi and project towards the intestinal lumen, thus allowing to increase the contact surface between the food bolus and the absorptive cells (JN et al., 2010). However, these villi are lost in the anatomical structure of the colonic mucosa during fetal development. On the other hand, invaginations of the epithelium appear in the connective tissue at the base of the villi and are called crypts of Lieberkühn, which contain the stem cells that allow the intestinal cellular renewal every 3 to 7 days (Barker, 2014). Crypts are the basic unit in the colon as opposed to the crypt/villus axis of the small intestine.

1.2 Intestinal barrier

The intestinal epithelium is the largest mucosal surface of the body protecting from exposure to the commensal microbiota contained in the GI lumen. Measuring approximately 400 m^2, this epithelium is composed of a wide layer of specialized and polarized cells that are interconnected through their membranes and with the basement membrane through protein complexes. Pluripotent stem cells generate a group of cells known as transit-amplifying cells, which divide successively and give rise to 4 main types of well-differentiated intestinal epithelial cells (IEC): goblets cells, Paneth cells, enteroendocrine cells and absorptive cells. These cells migrate towards the tip of the villus (or the apex of the crypt in the colon) and develop specific functions in the epithelium. Paneth cells are located at the bottom of the crypt and are involved in the epithelium defense (production of antimicrobial factors such as, α- / β-defensins and lysozyme) as well as in stem cells maintenance (Gassler, 2017). Goblet cells are responsible to synthesize and secrete mucus, while enteroendocrine cells produce hormones and neuropeptides with important functions which differ along the length of the GI tract. Finally, absorptive cells represent 80% of total epithelial cells and are adapted for metabolic and digestive functions, as well as the development of the innate immune response due to the expression of specific receptors at their surface (Pott and Hornef, 2012; Van Der Flier and Clevers, 2009).

Other IEC types that play an important role in the defense are Tuft cells that have activity against helminths and microfold (M) cells that are located in specific regions of the small intestine and known as aggregated lymphoid follicles or Peyer's patches. M cells are associated with immunological vigilance and maturation through the recognition of luminal antigens or microorganisms and their subsequent presentation to the underlying immune cells (Peterson and Artis, 2014). In general, IEC and their functions allow the formation of a dynamic physical and biochemical barrier protecting the host.

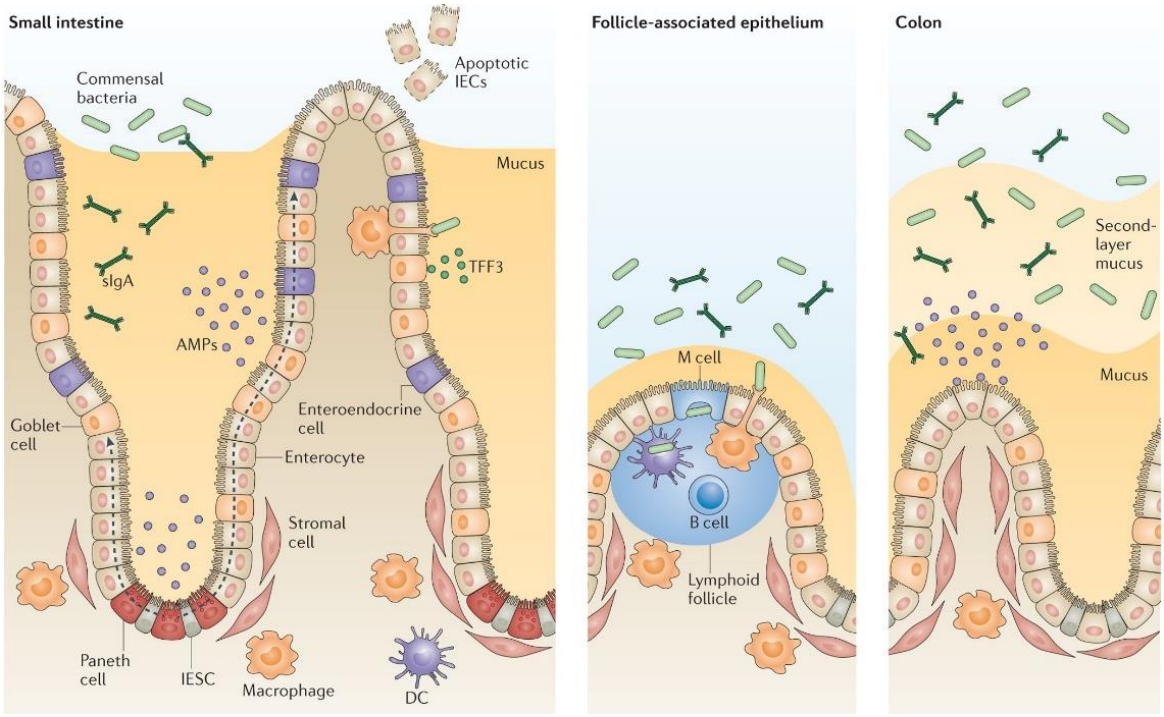


Fig.1: Composition of the intestinal epithelium. The intestinal epithelium contains a single layer of specialized cells (enterocytes, goblet cells, Paneth cells and enteroendocrine cells) that form a physical and biochemical barrier capable of separating the commensal microbiota from the immune system. These cells are continuously produced by the stem cells (IESC) found at the bottom of the crypt to proliferate, differentiate and migrate towards the tip of the villus (small intestine) or the crypt (colon), with the exception of Paneth cells. Goblet and Paneth cells belong to the secretory lineage and produce a group of factors (trefoil factor 3 or Tff3), proteins (mucins) and antimicrobial peptides (AMP). They constitute essential components of the mucus, a hydrogel that covers the epithelium and allows the exclusion of bacteria from the epithelial surface. Another essential factor in the mucus layer is secretory IgA (sIgA), which is synthesized by plasma cells and released into the intestinal lumen through a process of transcytosis. Microfold or M cells present in the region of the epithelium-associated lymphoid follicles are involved in the maturation of the immune system by sensing microbes and luminal antigens and transporting them across the barrier to dendritic cells and macrophages *(Peterson and Artis, 2014), with permission from Nature Reviews Immunology.*

1.2.1 Goblet cells and mucus

Goblet cells are responsible to produce the mucus that is further organized as layers to act as the first line of defense of the epithelial barrier. Specifically, in the colon the mucus is observed as a semi-sterile space of approximately 100 µm and covers the intestinal epithelium. With the help of its hydrophobic and surfactant properties, mucus main function is to limit and prevent bacteria attachment and invasion from the intestinal lumen towards epithelial cells (Qin et al., 2008). It is well accepted that the small intestine contains one single thin mucus gel layer while the colon harbors two distinct mucus layers. The inner layer, also called glycocalyx, is a stratified, denser and sterile region, which is related to the protection, hydration, renewal and differentiation of the epithelium while promoting oral

tolerance (Ugolev and De Laey, 1973; Shan et al., 2013). On the other hand, the external or loose mucus layer covers the feces and provides a source of nutrients to the commensal bacteria found in this region. Furthermore, this layer contains antimicrobial peptides, water and secretory immunoglobulin A (sIgA) synthesized by plasma cells located in the lamina propria (Mathias et al., 2015; Johansson and Hansson, 2016). The mucus concentrates these factors close to the epithelium and therefore generates an antibacterial gradient from the epithelial cells to the lumen. This could explain, to some extent, why bacteria are not in contact with epithelial cells of the small intestine. Also, the outer layer expands 2 to 3 times in volume due to the activity of endogenous proteases, which in turn increase the size of the pores, so that bacteria can penetrate it. However, this penetrability is a dynamic process given that goblet cells continually renew the mucus layer, a process that pushes bacteria towards the lumen. It might explain why many pathogenic bacteria have developed motility features among their morphological characteristics in order to be able to swim against the mucus flow to overcome the diffusion barrier. Hence the mucus thickness of the small intestine may thus be the reason why pathogenic bacteria mainly infect this region when compared to the whole GI tract (McGuckin et al., 2011). It is also proposed that the commensal microbiota, represented by more than 10^{14} bacterial cells and approximately a thousand of identified species, may influence the accessibility and penetrability of the mucus. However, the molecular mechanisms linked to this modulation have not been fully elucidated (Jakobsson et al., 2015).

The proportion of goblet cells along the intestine differs depending on the segment analyzed. Studies suggest a gradual increase throughout the epithelium, where the lowest being detected in the duodenum, while the highest number of goblet cells being in the colon (Karam, 1999). Moreover, different functional types of goblet cells are found in the intestine depending on their location within the small intestine or the colon (Gustafsson et al., 2012; Yu et al., 2010; Specian and Neutra, 1982; Johansson, 2012). The study of mucin turnover in the small intestine by *in vivo* labeling of O-glycans with N-azidoacetylgalactosamine, has revealed important differences in subpopulations of goblet cells in terms of rate for mucin biosynthesis and secretion. While, transmembrane MUC17 is similarly produced along the crypt-villus axis, the turnover of MUC2 is slower in crypt goblet cells when compared to those detected in the villi (Schneider et al., 2018).

Goblet cells derive from a common cell progenitor and their fate depends on the NOTCH signaling pathway. When the NOTCH pathway is activated, hairy and enhancer of split-1(HES1) is expressed, promoting an absorptive phenotype (Katoh and Katoh, 2007). However, the suppression of NOTCH triggers the rapid conversion of epithelial cells into cells of the secretory lineage, such as goblet cells, concomitant in turn with the activation of atonal homolog 1 (ATOH1 or MATH1) (Yang et al., 2001; Schonhoff et al., 2004; Gregorieff and Clevers, 2005; Radtke and Clevers, 2005; Kopan and Ilagan, 2009; VanDussen and Samuelson, 2010; Noah et al., 2011). Downstream of MATH1, other elements such as the growth factor independent 1 (GFI1), the SAM pointed domain containing ETS transcription factor (SPDEF) (Shroyer et al., 2005) and Krüppel-like transcription factor 4 (KLF4) (Zheng et al., 2009; Ghaleb et al., 2008), all contribute to goblet cell terminal differentiation.

The cytoplasm of goblet cells is occupied by a structure known as distended theca which contains granules. In these vesicles is present a family of large glycoproteins called mucins, which constitute the main component of the mucus. Mucins have a polymeric structure and are attached to numerous side chains of hygroscopic and hydrophilic oligosaccharides (O-linked-oligosaccharide). Glycans contribute to the sticky binding sites formation in the hydrogel, thus trapping microorganisms to facilitate their subsequent elimination (Barr et al., 2013). In humans, more than 20 different mucin types have been detected, which are encoded by mucin genes represented as MUC, followed by a number associated to the chronological order in which they were identified. Mucins are classified into two structural groups: membrane-associated (or transmembrane) mucins, among which are MUC1, MUC3B, and MUC3A, the latter being specific to the intestinal epithelium, and secreted mucins such as MUC5AC, MUC5B, MUC6, MUC19 and MUC2 (Dhanisha et al., 2018). The synthesis process of these mucins as simple polypeptides passes through the endoplasmic reticulum and the Golgi apparatus, where they undergo different post-translational modifications to complete their maturation, such as N/O-glycosylation, fucosylation and addition of other chemical groups such as sulfates or acetyls (Faderl et al., 2015; Thomsson et al., 2012; Matsuo et al., 1997).

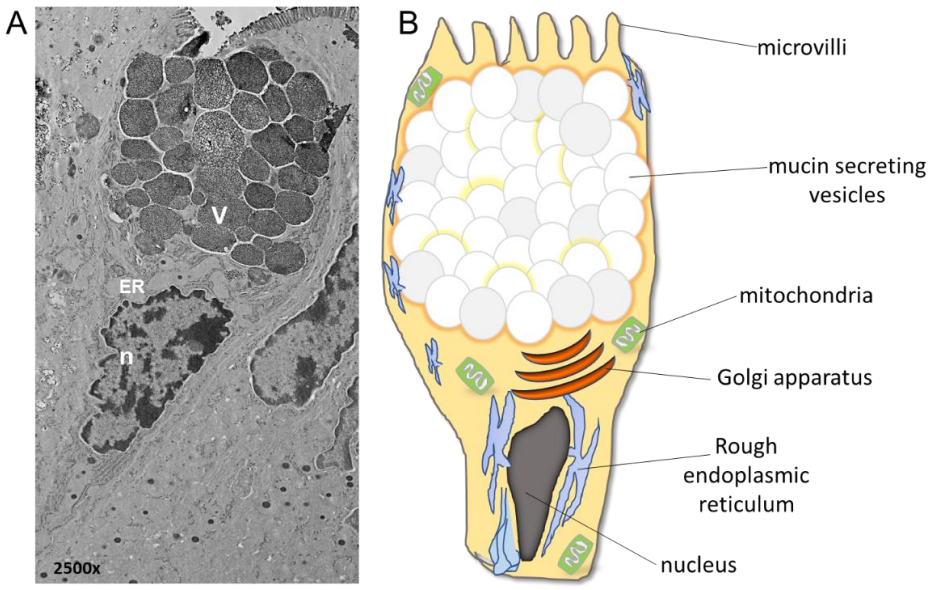


Fig. 2: Structure of intestinal goblets cells. A) Electron micrograph of ileal goblets cells. B) Schematic presentation of different cellular components. (microvilli, V: mucin secreting vesicles, mitochondria, Golgi apparatus, ER: rough endoplasmic reticulum, n: nucleus).

MUC2 constitutes the most abundant secreted mucin in the mucus of both the small intestine and colon and plays a determining role in the organization of the hydrogel layer (Johansson et al., 2008). Several studies suggest that MUC2 has approximately 5100 amino acids and is located within 29.5 kb DNA fragment, which is found at chromosomal locus 11p15.5 (Dekker et al., 2002; Allen et al., 1998). The monomers of this glycoprotein contain two highly glycosylated core domains known as proline, threonine, and serine-rich mucin domains (PTS domains), the cysteine-rich N- and C-terminal regions with 1300 and 1000 amino acids respectively, and four D-domains of the von Willebrand factor. All these regions are folded and stabilized from the presence of numerous disulfide bonds, resulting in a viscous network of gel-forming mucins. The rodent homologs of human MUC2 display a motif of similar structure (Lang et al., 2007; Birchenough et al., 2015). After being processed in Golgi apparatus, the MUC2 glycoprotein is densely packed into the secretory granules due to the low pH and the high concentration of Ca^{2+} in these compartments, to then be released by different routes: the constitutive (continuous secretion at basal levels) or stimulated Ca^{2+} -dependent pathways. The production and massive secretion of MUC2 by exocytosis during stimulated secretion can be initiated by different external stimuli such as hormones/neurotransmitters (prostaglandins, acetylcholine), microorganisms and their

products, cytokines (IL-22, IFN-γ) as well as reactive oxygen and nitrogen species. This process occurs through activation of secondary messengers such as Ca^{2+} (compound exocytosis that allows the fusion and release of multiple mucin granules) or cyclic adenosine monophosphate (cAMP) which induces the release of simple granules (Kim and Ho, 2010; Songhet et al., 2011; Turner et al., 2013). Recent advances suggest a connection between mucin secretion by compound exocytosis and the formation of goblet cell-associated antigen passages directly activated by the action of acetylcholine on muscarinic receptors of these cells (Knoop and Newberry, 2018). After being produced, the small intestine mucus is not attached to the epithelium but easily carried along the GI by peristalsis. This phenomenon coupled to the fast mucus renewal contribute to maintaining the protection of the villi due to elimination of bacteria from the surface, in addition to the fact that there is a high concentration of antimicrobial factors in this zone (Ermund et al., 2013; Vaishnava et al., 2011). However, this effect is not observed in germ-free mice where MUC2 remains bound to goblet cells. In order to release this mucin from the production site, the proteolytic enzyme meprin β needs to be activated. This metalloprotease is located at the enterocyte apical membrane and is activated when exposed to bacteria, which allows its release and diffusion in the mucus. Thus, it appears that there is a close relationship between the production-release mechanisms of mucus and the presence of bacteria in the small intestine (Schütte et al., 2014).

Other factors synthesized by goblet cells to ensure mucus quality are Fcg-binding protein (FCGBP) (directly linked to MUC2 to act as a cross-linker in the network of this protein), AGR2 (linked to goblet cell differentiation), CLCA1, the granulated zymogen 16 protein (ZG16) (linked to bacterial aggregation and prevention of diffusion into the mucus layer), Trefoil factor 3 (TFF3), and the resistin-like pro-inflammatory molecule RELMβ (Allaire et al., 2018). TFF3 is the second most abundant product in secretory vesicles and belongs to a family of small peptides highly expressed in the GI. Its activity is associated with structural integrity of the mucus and the protection and repair of epithelial tissues by inhibiting, for instance, apoptosis. This factor is also related to the migration of IECs and angiogenesis. Furthermore, it contributes to the innate immune response mediated by different receptors such as the Toll-like receptor family (Taupin and Podolsky, 2003; Podolsky et al., 2009; Kjellev, 2009). However, its antimicrobial activity has not been widely studied. In contrast, RELMβ is strongly induced apically during enteric infections such as in murine helminth

infection and IBD models. Its activity is linked to the chemotaxis inhibition of parasites. On the other hand, RELMβ participates in MUC2 secretion, T cells responses and during macrophages activation (Herbert et al., 2009; Bhinder et al., 2014; Nair et al., 2008). Thus, goblet cells contribute dynamically to the intestinal barrier functions. MUC2 is a crucial component for these functions and its depletion from the mucus layer contributes to the development of inflammatory diseases and cancer via permissive interactions between commensal bacteria and the epithelium (Velcich et al., 2002; Van der Sluis et al., 2006; Bergstrom et al., 2010; Tadesse et al., 2017; Johansson et al., 2014).

1.2.1.1 Antimicrobial components in the mucus

Goblet cells are not the only entities to ensure defense mechanisms and protection of the host against microorganism invasions. Other epithelial cell lineages such as enterocytes and Paneth cells are involved and produce a number of components with antimicrobial properties. Although enterocytes are capable of synthesizing some antimicrobial peptides (AMP), Paneth cells of the small intestine are the major source for the production of these molecules. These cells are characterized with granules filled with peptides and enzymes such as lysozyme, cathelicidins, α-defensins (cryptdins in mice), phospholipase A2 ($sPLA_2$), etc, (Gallo and Hooper, 2012; Bevins and Salzman, 2011; Bel et al., 2017). AMP induce bacterial lysis due to pore formation in the membrane of these microorganism. On the other hand, specific enzymes such as cryptdin 3 cause an increase in water secretion within the intestinal lumen, allowing physical detachment of bacteria near the epithelial surface. Furthermore, α-defensins (more than 20 cryptdin molecules in mice) are related to the adaptive immune response (Chu et al., 2012; Elphick and Mahida, 2005; Lencer et al., 1997). Other AMP such as REGIII (γ, β) molecules that belong to the C-type lectin family cause destabilization of the Gram-positive bacterial membrane by direct binding to phospholipids. More specifically, REGIIIγ has the main function of keeping the small intestinal epithelial surface sterile and free of bacteria (Vaishnava et al., 2011; Mukherjee et al., 2014).

sIgA is a fundamental molecule of the GI immune response and is produced by plasma cells of the lamina propria. sIgA synthesis contributes to the defense against intestinal pathogens and the control of the commensal microbiota, based on its ability to sequester antigens and microbes through a process known as immune exclusion. Murine models made deficient in sIgA synthesis showed a significant imbalance in the composition of the commensal bacterial

community. These observations suggest an important role for these antibodies in modulating the diversity and quantity of bacteria in the intestine. It has also been proposed that the highest sIgA response is obtained when bacteria reach the inner mucus layer, this being a powerful indication of subsequent penetration/invasion through the intestinal mucosa (Palm et al., 2014; Suzuki et al., 2004). sIgA is secreted to the mucosal surface as polymeric antibodies under stable conditions, more specifically as immunoglobulin dimers that interact with the polymeric immunoglobulin receptor (pIgR) on its way through epithelial cells toward the lumen. The expression of this receptor in the intestine can be regulated by cytokines such as IL-17 or by activation of signaling pathways such as NF-κB. Two antibody subclasses are distinguished in humans: IgA1 and IgA2, the latter being more stable to bacterial proteolytic degradation and therefore predominant in the mucosa (Cerutti, 2008; Mathias et al., 2015; Brandtzaeg, 2013; Pabst and Slack, 2020).

In summary, these multiple secretory strategies emerging from the epithelial barrier contribute to limiting or resolving pro-inflammatory events and to maintaining the integrity and a healthy state of the intestinal epithelium.

1.2.2 The junctional complexes as a physical barrier of the intestinal epithelium

The epithelial barrier controls ions, nutrients and water passage from the lumen to the organism and restrict translocation of luminal antigens such as microorganisms and their harmful derivatives. Transport in the epithelium occurs by three mains pathways: the transcellular pathway, the transporter-mediated pathway and the paracellular pathway. The fundamental characteristic of the paracellular pathway is to allow the passage of molecules by passive diffusion between the spaces of adjacent cells. Precisely, the function of the narrow physical barrier that controls and regulates the paracellular transport of various molecules is determined by a protein complex that forms the so-called intercellular junctions, made up of the tight junctions (TJ) in the most apical and lateral areas of the cells, followed by the adherens junctions (AJ), the desmosomes and the Gap junctions.

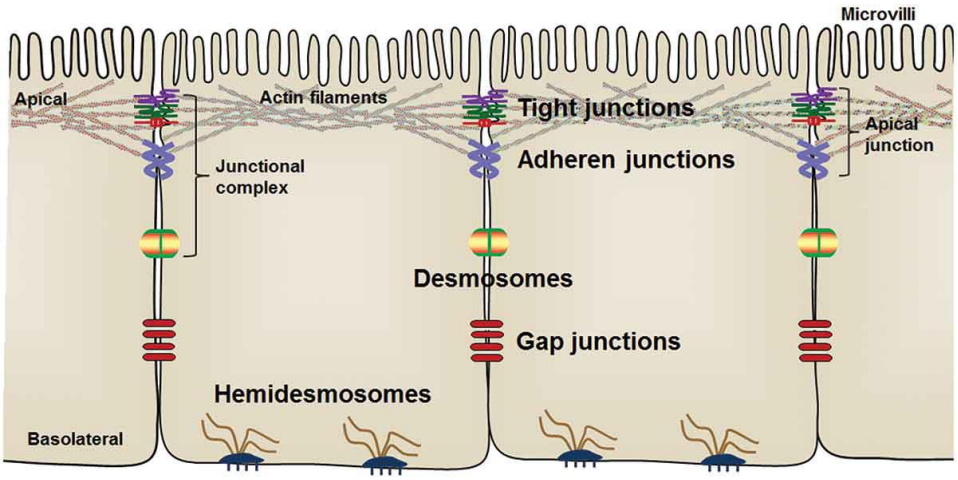


Fig. 3: Distribution of the intercellular junctional complex among intestinal epithelial cells. Tight junctions are located in the most apical and basolateral area of IECs and are responsible for sealing the epithelial barrier. Adherens junctions interconnect with actin filaments, participate in cell signaling processes and, like the desmosomes, maintain cohesion and mechanical adhesion between cells. Hemidesmosomes connect the cell intermediate filaments to basal lamina, allowing cell anchoring and the interactions with other proteins. Gap junctions form tunnels between the cells for ions and water molecules transport *(Zhu et al., 2018), with permission from Taylor & Francis.*

1.2.2.1 Tight junctions

TJ are formed by the assembly of a group of three families of cytosolic and transmembrane proteins: the Claudins (CLDN), the occludins (OCDLN), and the junctional adhesion molecules (JAM) (Luissint et al., 2016). In addition to limiting selective traffic through the paracellular barrier of the epithelium, TJ determine the correct polarity of IECs. They form a dynamic multiprotein complex programmed to open and seal the barrier during normal or threatening conditions from the environment. Among the components of TJ are the zonula occludens protein family members (ZO 1-3) and cingulin, whose functions are related to TJ stabilization via interaction with cytoplasmic proteins of the peripheral membrane and the actin cytoskeleton (Cereijido et al., 2007). Similarly, other proteins such as JAM, have a central role in the regulation of the epithelial barrier, migration and cell proliferation. While occludins have not been widely studied and some controversy exists in regard to their functional activity, they are involved in signal transduction processes and in strengthening the structural properties of TJ (Al-Sadi et al., 2010; Luissint et al., 2014 ; Luissint et al., 2016; Zhu et al., 2018).

TJ are mainly made up of CLDN, which play a key role in maintaining the paracellular barrier function. This family of approximately 27 isoforms, at least identified in mammals, are integral membrane proteins with unique characteristics in terms of expression and

distribution throughout the epithelial tissues (Cording et al., 2013). Many of the CLDN members have tensile or selective barrier-forming properties, allowing cell stability (CLND1, 3, 4, 5, 11, 14 and 18), while others are classified as leak CLDN (CLDN2, 10, 15 and 17), because they increase paracellular permeability, from the formation of channels or pores that allow the anions, cations, and water passage (Lee, 2015; Günzel and Yu, 2013). Different studies show that CLDN composition varies depending on age and the GI tract segment analyzed. Whereas some isoforms such as CLDN2 and CLDN15 are expressed mainly at the crypt base, others such as CLDN3, CLDN4, and CLDN7 are localized toward the luminal zone. Some studies suggest an apparent dissociation or alteration in the intestinal CLDNs during the development of inflammatory events, thus highlighting the importance of CLDNs for optimal IEC barrier function (Capaldo and Nusrat, 2015; Zeissig et al., 2007; Findley and Koval, 2009).

1.2.2.2 Adherens junctions

The AJ located below the TJ maintain mechanical bonding between adjacent cells and participate in the cellular signaling processes leading to the regulation of gene transcription (Takeichi, 2014). This adhesion is maintained by a transmembrane proteins complex including cadherins and nectins, which interact at the same time with other anchoring proteins (α- and β-catenins) while making a bridge with the actin filaments (Ivanov and Naydenov, 2013). E-cadherin constitutes one of the most important molecules among the members of the AJ. Its intestinal epithelial functions are not only limited to the cellular adhesive properties and desmosomes formation, but also impact cell migration and proliferation through the Wnt signaling in the intestinal crypt cells (Nelson and Nusse, 2004; Groschwitz and Hogan, 2009). Both TJ and AJ are regulated by kinases or phosphatases that induce phosphorylation events, thus controlling the interaction with other proteins and their enzymatic activity (Bertocchi et al., 2012; Garcia et al., 2018).

The imbalance in the intercellular junctional organization and therefore, a loss of the intestinal barrier functionality can lead to the appearance of different diseases, one of the most studied being inflammatory bowel diseases (IBD).

1.3 Inflammatory Bowel Diseases

IBD are a group of chronic disorders that cause inflammation of the GI tract. IBD are mainly represented by Crohn's disease (CD) and ulcerative colitis (UC), which are characterized with periods of active and remission phases. UC is characterized by diffuse inflammation of the colonic mucosa with the most common symptoms being bloody diarrhea. On the other hand, CD causes ulceration of different parts of the intestinal tissue, especially at the level of the terminal ileum and colon. CD is characterized by symptoms of diarrhea, abdominal pain, and weight loss. Although IBD can be diagnosed at any age, a huge majority of new cases are detected during adolescence or early adulthood (De Souza and Fiocchi, 2016).

The incidence of IBD is high worldwide with a higher prevalence in Western countries. Nevertheless, many newly industrialized or increasingly developing countries in Asia, Middle East, Africa, and South America are experiencing a rapid increase in the incidence of these diseases (Kaplan and Ng, 2017). Canada has one of the highest incidence/prevalence rates. In 2018, approximately 270,000 individuals were reported to be living with IBD in the country and this number could rise to approximately 400,000 by 2030. Although there are advances in elucidating the mechanisms that influence the development of these diseases, there are currently no accurate treatments that allow the cure of patients affected by IBD.

The etiology or pathogenesis of IBD is attributed to the presence of complex interactions among a group of factors including genetic predisposition of the host, dysregulated immune response to environmental triggers (diet factors, alcohol, antibiotics, smoking, stress) and imbalance in the dynamic behavior of the commensal microbiota, known as dysbiosis (Abraham and Cho, 2009).

1.3.1 Intestinal barrier dysfunction in IBD

There is now clear evidence that integrity of the intestinal epithelial barrier is important in the development of IBD, since it constitutes the delimiting factor for exposure of the microbiota to the host immune system. Consequently, defects in intestinal mucosa homeostasis can trigger alteration of intestinal permeability. This will lead to antigens translocation and activation of signaling cascades causing apoptosis, erosion, and ulceration, which are considered as crucial steps in the initiation or development of chronic intestinal inflammatory disorders (Mankertz and Schulzke, 2007; Antoni et al., 2014; Zeissig et al., 2004; Schulzke et al., 2006). It has been shown that in UC or CD patients, there is an altered

expression of the proteins that make up the TJ (Takeuchi et al., 2004; Hollander, 1988; Schmitz et al., 1999; Prasad et al., 2005).

CLDN1, among the most studied CLDNs, regulates intestinal homeostasis and modulates the activation of the NOTCH signaling pathway. There are growing evidences that suggest a significant increase in the expression of CLDN1 in murine tissue in the presence of active inflammation, leading then to the activation of NOTCH and therefore the inhibition of goblet cells differentiation with the result of reducing MUC2 production and mucus formation (Poritz et al., 2011; Pope et al., 2014). Precisely, Gowrikumar *et al*. proposed that CLDN1 in IBD patients may constitute a possible marker of severity in colitis and malignancy by promoting susceptibility to colitis-associated cancer (Gowrikumar et al., 2019). Other studies suggest that in the intestinal mucosa of patients who develop CD, there is a change in the expression pattern of CLDN3, -5, and -8 (sealing process) as well as for the occludin protein (Zeissig et al., 2007; Goswami et al., 2014). Similarly, in UC, a decrease and delocalization of some TJ proteins, such as occludin, CLDN3, -4, and -7 was reported (Prasad et al., 2005; Oshima et al., 2008; Heller et al., 2005; Weber et al., 2008). Deficiency in CLDN7 results in dysregulation of the paracellular flow of small molecules such as microbial products (bacteria-derived N-formyl-L-methionyl-L-leucyl-L-phenylalanine peptides), causing inflammation in murine models. It also promotes the activation of certain metalloproteinases activating in turn signaling events such as the NF-κB pathway to finally induce the inflammatory process and the loss of cell-extracellular matrix interaction (Tanaka et al., 2015; Ding et al., 2012). On the other hand, CLDN12 was shown to be induced in IBD patients, especially into the ileum of CD patients (Lameris et al., 2013). This protein is involved in vitamin D-dependent Ca^{2+} permeability in the intestinal epithelium (Fujita et al., 2008). Additionally, CLDN18 exhibits higher expression in tissues samples of UC patients, as well as in mice during experimental colitis (Zwiers et al., 2008; Dotti et al., 2017). Strand, an integral membrane protein of approximately 24.5 kDa, influences cell permeability and transepithelial electrical resistance (TER) due to the formation of channels allowing the transport of water and small cations such as Li^{+}, Na^{+}, and K^{+} (Rosenthal et al., 2010; Weber et al., 2015). CLDN2 is mostly expressed in the GI epithelial tract in both humans and rodents at birth. However, its expression decreases rapidly afterward (Tamura et al., 2011; Holmes et al., 2006; Ong et al., 2020). CLDN2 becomes highly expressed during intestinal

inflammation and IBD accordingly to the severity of the disease (Lameris et al., 2013; Hering et al., 2012; Luettig et al., 2015). Increase expression of CLDN2 can trigger the alteration of the TJ, leading to the loss of solutes and fluids through the epithelial barrier, the appearance of symptoms such as diarrhea, and ultimately antigens translocation. Recently, Raju *et al*. showed that inactivation of the channels formed by CLDN2 attenuated immune-mediated colitis in mice (Raju et al., 2020).

Several studies indicate that cytokines also contribute to alteration of the intestinal epithelial barrier during IBD. Indeed, IL-13, TNFα and IFN-γ can stimulate expression of CLDN2 in *in vitro* models and reduce expression of other barrier-forming proteins such as CLDN1, -5, -7 or ZO-1 (Mankertz et al., 2009; Weber et al., 2010; Amasheh et al., 2009; Heller et al., 2005). In response to these inflammatory mediators, specific epithelial barrier disorders occur in association with TJ proteins trafficking in the membrane. For example, TNFα and INF-γ can activate the selective endocytosis phenomenon of occludin, CLDN1, CLDN4 and JAM-A (Utech et al., 2010; Bruewer et al., 2005; Marchiando et al., 2010). It was also observed that IL-6, an important pro-inflammatory mediator produced by both immune and epithelial cells, as well as IL-17, induce CLDN2 expression by activating different signaling pathways such as MEK/ERK or PI3K (Kinugasa et al., 2000; Suzuki et al., 2011). On the other hand, TJ assembly depends on myosin light chain kinase (MLCK) that is affected in IBD patients (Blair et al., 2006). MLCK activation and phosphorylation stimulates the redistribution of TJ proteins into subcellular compartments within enterocytes. Induced expression of MLCK caused by TNFα and IL-1β via the activation of NF-κB results in the enhancement of cell permeability as determined by *in vitro* assays (Ye and Ma, 2008; Al-Sadi et al., 2010). Both cytokines also promote the cleavage of other proteins belonging to the AJ and desmosomes, such as desmocholine 2 or E-cadherin. The latter represents a potential biomarker given that its proteolytic fragments can be detected in the inflamed mucosa of UC patients and experimental colitis models in mice (Kamekura et al., 2015; David and Rajasekaran, 2012).

Defects in the mucus layer can trigger the appearance of IBD. Polymorphism in some *MUC* genes that code for members of the mucin family have been shown to be in association with the pathogenesis of the disease (Moehle et al., 2006; McCole, 2014; Kyo et al., 2001). In addition, loss of functional MUC2 in mice leads to spontaneous appearance of colitis (Van

der Sluis et al., 2006; Heazlewood et al., 2008). Interestingly, Visschedijk *et al*. also observed the development of UC cases associated with the production of MUC2 rare variants (Visschedijk et al., 2016).

A decrease in the thickness and uniformity of the mucus layer in UC patients favor commensal microorganisms or their metabolite to come into contact with the epithelium. This can be due to the loss of goblet cells, as well as with the attenuation of their secretory functions that contribute to meeting microbial challenges. Besides, variations in secondary modifications of structural components of the hydrogel network, such as, for example, O-glycosylation or detection of Shorter O-linked oligosaccharide side chains in mucin can impact the quality of the gel. These changes have been correlated with the extent of the inflammatory status and disease activity in the intestine of UC patients (Longman et al., 2006; Strugala et al., 2008; Larsson et al., 2011; Johansson et al., 2014; Fu et al., 2011; Alipour et al., 2016; Van Der Post et al., 2019; Dotti et al., 2017). On the other hand, although a hyperproduction of mucins was confirmed in CD patients, changes in processes of glycosylation and sulphation or gel hydration were proposed as mechanisms contributing to the decrease in viscosity, greater bacterial penetrability and erosion of the mucus layer (Buisine et al., 2001; Abraham and Cho, 2009; Sun et al., 2016). Undoubtedly, the integrity of the mucus barrier constitutes a turning point in the protection and repair of the epithelium during inflammation. However, it is not entirely clear whether such defects are the cause or the consequence of a dysregulated immune system during the initiation of IBD.

1.3.2 Microbiota in IBD

The human intestine is colonized by thousands of microorganisms, mostly of bacteria, which establish mutual relationships by forming a complex microbial ecosystem. This symbiotic interaction between the host and commensal microbes and their metabolites will influence both the intestinal environment, as well as the physiology of many tissues and organs in the human body (Sender et al., 2016). Several evidences support that the intestinal microbiota plays an essential role in various processes such as the maturation of the immune system, the regulation of the neuroendocrine and nervous system response, as well as the initiation and development of various diseases (Heijtz et al., 2011; Uronis et al., 2009; De Filippo et al., 2010). Different studies have demonstrated the beneficial role of these microorganisms for human health. For example, some bacterial species can synthesize a group of essential

nutrients, such as vitamins, which are usually obtained through food (Magnúsdóttir et al., 2015). They can also degrade non-digestible materials from the diet, which generates a set of metabolites such as short-chain fatty acids (SCFA) with multiple positive effects on the body (Gill et al., 2018). In addition, under normal physiological conditions, the intestinal microbiota contributes to energy production in the host organism, integrity of the mucosa and also prevention against pathogenic microorganisms colonization.

The GI tract is relentlessly challenged by the luminal contents harboring innumerable microorganisms and food antigens (Rapozo et al., 2017). Also, genetic variation in the host can affect the microbiome towards dysbiosis (Hall et al., 2017; Imhann et al., 2018). Interestingly, alterations of the gut microbiota are seen in several intestinal adverse conditions, including irritable bowel syndrome, chronic idiopathic diarrhea, celiac disease, and IBD (Shanahan, 2007; Swidsinski et al., 2008; Quigley, 2011; D'Argenio et al., 2016; Eck et al., 2017). The persistence of pathogens in the intestine, an abnormally permeable mucosal barrier leading to excessive bacterial translocation, and the presence of dysbiosis in the commensal microbiota are some of the hypotheses raised to explain the pathogenesis of these diseases (De Hertogh et al., 2008; Kalischuk and Buret, 2010; Liu et al., 2020). Much of the evidences supporting a functional role of the microbiota in the development of chronic inflammatory disorders are from work with animal models, more specifically in germ-free or antibiotic-treated conditions. In germ-free mice with chemical or genetically induction of inflammation, the impact of the bacterial presence was exposed on the appearance or manifestation of symptoms similar to those found in IBD, when rodents were grown under conventional conditions (Khan et al., 2019). It is argued that dysbiosis is a cause as well as an outcome of IBD. Dysbiosis is characterized as the flowering of pathobionts and the loss of commensals or diversity, key elements to be considered in the search of mechanisms and therapies for IBD (Levy et al., 2017; Kostic et al., 2014).

Quantitative and qualitative changes in the composition of the gut microbiota was detected in CD and UC (Alam et al., 2020; Yilmaz et al., 2019; Lloyd-Price et al., 2019). A reduction of up to 25% of microbial genes was observed in IBD patients compared to healthy individuals (Qin et al., 2010). These microbial changes were mostly related to the loss of beneficial species belonging to genera such as *Firmicutes* or *Bacteroides*, or families such as *Lachnospiraceae* and *Ruminococcaceae*. In parallel, a relative increase in harmful bacterial

species belonging to *Enterobacteriaceae* (*Escherichia coli*) was showed (Chassaing and Darfeuillemichaud, 2011; Hedin et al., 2014; Hedin et al., 2016; Nguyen, 2011; Bien et al., 2013; Li et al., 2014; Gilbert et al., 2016). From metabolomic and taxonomic profile analysis of microbial communities in human feces from IBD patients, Franzosa *et al*. correlated a loss of taxonomic diversity with depletion of enzymes, chemicals, or differentially abundant metabolites during the manifestation of intestinal dysbiosis (Franzosa et al., 2019).

1.3.2.1 Intestinal microbiota and metabolites modulate the development and function of the immune system and play a critical role in IBD.

SCFA such as butyrate that are produced by intestinal bacteria regulate protective immunity and reduce tissue inflammation. A decrease in the expression and secretion of SCFA, which impair the metabolism of tryptophan, was related to the loss of the integrity of the intestinal barrier in IBD (Schirmer et al., 2019). Bacteria can metabolize tryptophan from the diet. Tryptophan metabolites can in turn interact with the aryl hydrocarbon receptor and attenuate inflammation. Wlodarska *et al*. reported that IBD patients have low levels of indoleacrylic acid (bacterial indole metabolites), whose function is to regulate mucus production and suppression of inflammatory cytokines (Wlodarska et al., 2017). Similarly, a bacterial imbalance from the reduction of strict anaerobic species, such as *Clostridium* groups IV and XIVa, against the expansion of aerobic or facultative aerobic bacteria, can lead to an intraluminal increase in oxygen and therefore the development of metabolic changes (inflammation) in the intestine. It is known that these Clostridium species are capable of generating antigenic signals such as retinoic acid that contributes to the suppressive immune response and anti-inflammatory activity from the induction of regulatory T cells dependent on transforming growth factor-β (TGF-β), whose activation it is essential for intestinal homeostasis (Mucida et al., 2007; Albenberg et al., 2014; Atarashi et al., 2013; Furusawa et al., 2013). Some other species with great anti-inflammatory potential were also found affected in the commensal microbiota of UC or CD patients. For example, *Roseburia*, *Faecalibacterium prausnitzii* are linked to IL-10 secretion and the production of a 15 kDa protein with possible inhibitory properties of inflammation by suppressing the NF-κB pathway; *Bacteroides fragilis* synthesizes the immunomodulatory capsular polysaccharide A (PSA); sphingolipids protect against colitis episodes in mouse experimental models, and

finally *Akkermansia muciniphila* induces antigen-specific T cell responses and homeostatic IgG production (Liu et al., 2020; Guan, 2019).

1.3.2.2 Other bacteria associated with IBD

Microbes can reduce immune tolerance, leading to inappropriate activation of pathways designed to protect against pathogens invasion. Among the potentially pathogenic microbiota microorganisms related to IBD is the invasive proteobacterium *Campylobacter concisus*, which inhabits naturally in the oral cavity but can infect the GI tract by damaging the intestinal epithelial barrier through the production of cytokines such as TNFα (Mahendran et al., 2011; Mahendran et al., 2016). Another bacterium widely enriched in the mucosa of UC patients is *Fusobacterium varium*, whose mechanism of action is to invade IECs, a key event in triggering the inflammatory response (Sekizuka et al., 2017). Likewise, an abundant presence of *Ruminococcus gnavus* was detected in IBD patients. *R. gnavus* synthesizes and expresses an inflammatory glucorhamnan polysaccharide and also produces and metabolizes sialic 2,7-anhydro-Neu5Ac. This allows a better adaptation and nutritional competitive advantage of this microorganism in the mucus, due to its ability to break down mucins when compared to other bacteria (Nishino et al., 2018; Henke et al., 2019; Bell et al., 2019). The enterotoxigenic *Bacteroides fragilis* is also linked to IBD and colorectal cancer development. It promotes cell proliferation, the cleavage of E-cadherin that leads to increased mucosal permeability, and finally the secretion of pro-inflammatory interleukins (Zamani et al., 2017; Rhee et al., 2009). In addition, the invasive and adherent *Escherichia coli* (AIEC) is among the most studied microorganisms with the highest prevalence in samples from CD patients (Darfeuille-Michaud, 2002; Desilets et al., 2016; Prorok-Hamon et al., 2014). This bacterium colonizes the intestinal mucosa via the adhesin FimH localized at the end of the bacterial pilus or by invasion through type 1 pili, a crucial virulence factor for the recognition of the Carcinoembryonic antigen-related cell adhesion molecule 6 receptor expressed on IECs. Furthermore, AIEC can penetrate the epithelium via the M cells localized on the surface of the Peyer's patches, thus interacting with the immune cells of the lamina propria and inducing the inflammatory response. The infection, replication, and survival of this pathogen in macrophages allow the secretion of high levels of TNFα without inducing cell death (Lee et al., 2019; Palmela et al., 2018). On the other hand, some authors suggest the predominance and risk of suffering from chronic inflammatory processes, mainly UC, after successive

repetitions of infection with *Salmonella*. In a study carried out by Tripathi *et al*. a 79.7% positivity rate for specific *Salmonella* sequences in UC patients was identified by PCR compared to the 16.3% observed in healthy individuals (Tripathi et al., 2016). This suggests that the prevalence of *Salmonella* can be linked to the development of IBD. Similar conclusions were raised using *Salmonella* Typhimurium as a model of infection in mice made deficient for IL-10 or exposed to DSS during experimental colitis (Schultz et al., 2018). As additional pathogens linked to IBD, sulfate-reducing bacteria obtain their energy by oxidizing organic compounds or hydrogen while reducing sulfate to hydrogen sulfide (H_2S), which promote cleaving the disulfide bond in mucins and therefore favoring bacterial translocation (Ijssennagger et al., 2016). Some of these bacterial populations, such as *Desulfovibrio* species, are overexpressed in samples of the intestinal mucosa and feces from IBD patients (Mills et al., 2008; Verma et al., 2010; Loubinoux et al., 2002; Rowan et al., 2010). Finally, there are similar evidences to support a role for mycobiota dysbiosis in the pathogenesis of IBD. Specifically, *Candida albicans* were found to be abundantly expressed in CD and UC patients (Li et al., 2014; Mar et al., 2016).

1.3.3 Genetic variants and IBD

Genome-wide association studies (GWAS), as well as meta-analysis studies have become successful tools for the study of complex diseases including IBD. There are now more than 240 genetic risk loci identified for IBD, including some that are shared between CD and UC (De Lange et al., 2017; Uhlig and Muise, 2017). The clustering of these genes or loci involved with the onset or progress of these diseases shows important links with specific pathways including immune response, autophagy, cellular metabolic processes, defense, repair and intestinal epithelium barrier function (Khor et al., 2011; Graham and Xavier, 2020) (Fig. 4). Among the most studied genes are *NOD2*, *ATG16L1*, *IRGM*, *IL-23R*, *CARD9*, *RNF186*, *IL-17RA*, and *PRDM1*, with many of them containing several variants associated with risk or protection for the diseases (Uniken et al., 2017).

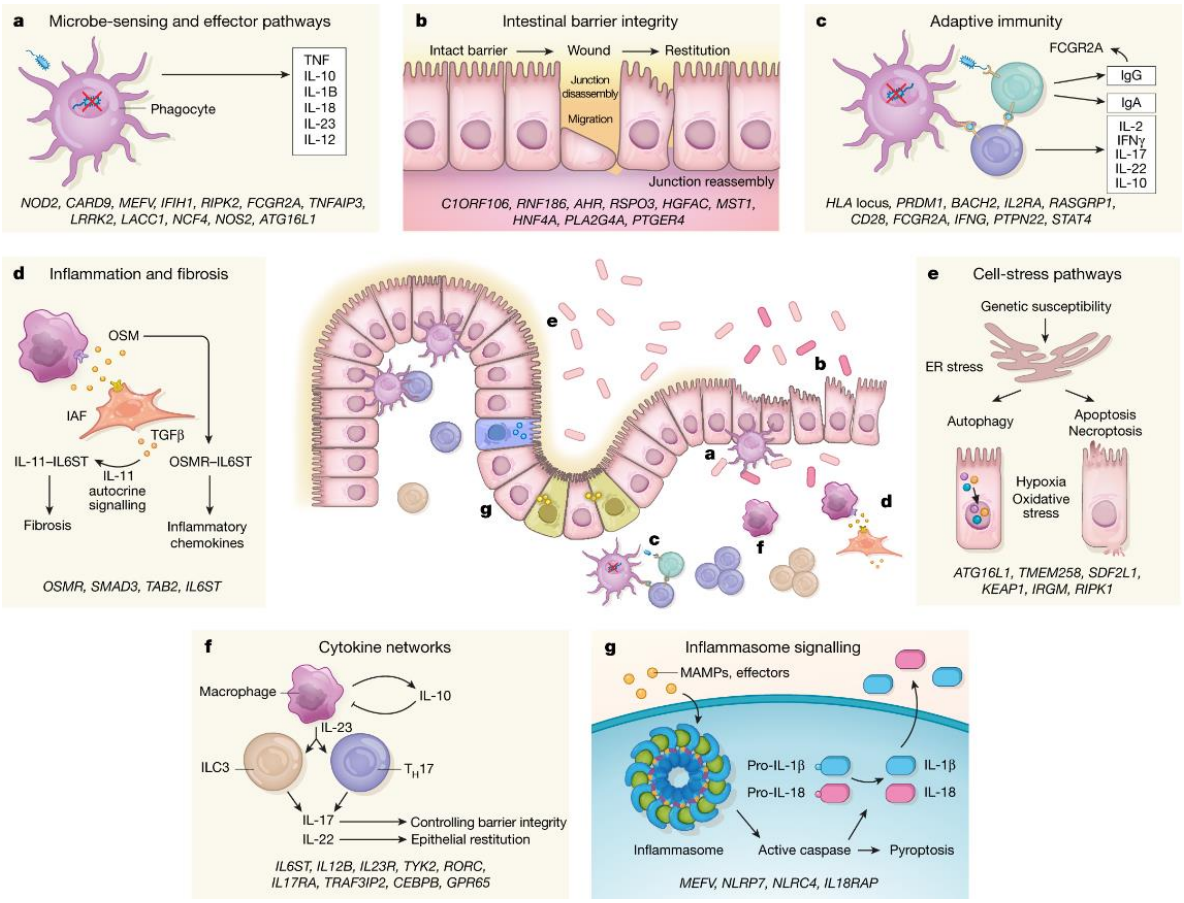


Fig. 4: Different pathways and genes identified as risk factors in IBD pathology. A) Sensing of microorganisms by phagocytes that induce the activation of cytokines or oxidative stress genes capable of promoting inflammation. B) The intestinal epithelial barrier, the complex of cell junctions and genes involved in the mechanisms of repair and maintenance of cell integrity. C) The adaptive immune system (B, T and dendritic cells) that trigger the specific or memory response against microorganisms. D) Disturbance in fibroblast cells that contributes to the dynamic response to inflammatory events. E) Pathways and cellular stress factors (hypoxia, autophagy) related to inflammation and cell death pathways. F) Cytokines that regulate the interconnection between stromal cells, cells of the immune system and IEC. G) Formation of the inflammasome complex through the recognition of antigens or extracellular microorganisms and activation of pro-inflammatory cell death pyroptosis *(Graham and Xavier, 2020), with permission from Nature.*

1.3.3.1 Intestinal barrier genes and IBD

Some works suggest a strong association between genetic markers involved in the formation, maintenance, and regulation of the intestinal epithelial barrier and the etiopathology of IBD. Understanding of the mechanisms involved in these processes could allow a better diagnosis and classification of patients and the application of possible treatment therapies. *CDH1* is associated with UC and encodes for E-cadherin, a component of AJ (McGovern et al., 2010). *CDH1* locus risk haplotypes rs12597188, rs10431923, and rs9935563 are associated with CD and lead to the accumulation of a truncated form of E-cadherin in the cytoplasm of IECs

(Muise et al., 2009). *GNA12* (rs798502), which codes for the Gα12 protein, is also related with UC. This protein is a GTPase that is bound to the cellular membrane and interacts with accessory proteins such as ZO-1 to promote correct TJ assembly (Anderson et al., 2011; Meyer et al., 2002). Protein tyrosine phosphatase nonreceptor type 2 gene locus 18p11 variants (*PTPN2*; rs2542151, rs7234029) are associated with CD. A possible *PTPN2*-mechanisms of action in the disease is through the strong activation of transcriptional agents such as NF-κB (Consortium et al., 2007; Barrett et al., 2008; Weersma et al., 2009; Glas et al., 2012). IBD genetic associations were also made for genes that code for the mucins. For example, the *MUC3* gene as well as rare alleles of the *MUC3A* gene inserted with an unusual number of 51bp repeat units were both associated with UC and CD (Kyo et al., 2001). A single nucleotide polymorphism (rs3180018 at 1q22) within the *MUC1* gene was also confirmed as a risk locus for CD. Furthermore, overexpression of MUC1 with an hypoglycosylation status bringing more susceptibility to degradation was observed in IBD patients (Franke et al., 2010). Finally, Vancamelbeke *et al.* carried out a study to determine the high or low polygenic risk of association with CD or UC. From a set of 128 genes related to the intestinal barrier, they observed an enrichment of some of these genes in IBD samples. In addition to the modulated expression of *HNF4A*, they confirmed an important dysregulation of many genes of the epithelial barrier in the ileum and colon during the active phase of UC and CD, including *CLDN1* and *CLDN8*. They also identified significant modulations in mucosal layer genes such as *MUC1*, whose dysregulation was also maintained during the inactive phase of the diseases (Vancamelbeke et al., 2017).

1.4 *HNF4A* as a susceptibility gene in IBD and its controversial role in the control of the intestinal epithelial barrier.

HNF4A is a single gene located on chromosome 20 in humans and encodes the transcription factor hepatocyte nuclear factor 4-α (HNF4α). This transcription factor (TF) is a member of the superfamily of ligand-dependent nuclear receptors (NR2A1) and has twelve isoforms, divided into P1 or P2 promoter-driven isoform classes (Babeu and Boudreau, 2014; Bridgham et al., 2010). HNF4α is expressed in multiple organs including the liver, pancreas, stomach, kidneys and the epithelium of the whole intestine (Drewes et al., 1996; Jiang et al., 2003; Sladek et al., 1990). Many functions have been attributed to HNF4α such as lipid and glucose metabolism, regulation of cell proliferation and differentiation and regulation of key

components of cell junctions (Battle et al., 2006; Hayhurst et al., 2001; Babeu and Boudreau, 2014; Lili et al., 2016). Although its functions have been extensively studied in the liver, HNF4α was also showed to play an essential role in the morphogenesis of the GI tract. *Hnf4a*-null mice showed high lethality during embryogenic development with severe defects in visceral endoderm formation (Chen et al., 1994). Additionally, it was observed that *Hnf4a* deletion in the mouse embryonic colon led to disrupted crypt topology characterized by a decreased number of epithelial and mature goblet cells (Garrison et al., 2006). Some studies have linked HNF4α with IBD. A significant reduction in intestinal HNF4α levels was observed in both UC and CD human samples (Ahn et al., 2008; Darsigny et al., 2009). Coincidently, mice conditionally deleted for *Hnf4a* in the intestinal epithelium were found to be more sensitive to experimental colitis when exposed at young age (Ahn et al., 2008), while displaying signs of spontaneous chronic inflammation resembling human IBD at older age (Darsigny et al., 2009). Subsequently, GWAS studies identified the *HNF4A* gene locus as being significantly enriched from UC cohorts (Barrett et al., 2009; Van Sommeren et al., 2011; Jostins et al., 2012; Chellappa et al., 2016). Genetic variations were also detected in the P2 promoter of *HNF4A* (rs1884613) in association with childhood-onset of CD (Marcil et al., 2012).

HNF4α interacts with genes that participate in the formation of a narrow intestinal epithelial barrier and that are functionally involved in IBD. Parviz *et al.* showed that the loss of HNF4α in the fetal liver induces an important reduction in E-cadherin expression (Parviz et al., 2003). In contrast, colonic epithelial loss of HNF4α did not influence E-cadherin expression (Garrison et al., 2006). Using a tamoxifen-inducible *Villin*-Cre;*Hnf4a*$^{fx/fx}$ conditional knockout mouse model, Cattin *et al.* showed an increase in IECs proliferation in association with the activation of Wnt/β-catenin signaling pathway (Cattin et al., 2009). They also reported an increase in intestinal permeability in these mutant mice. Coincidently, they observed a decrease in the expression of *ZO-1*, *Cldn4*, and *Cldn7* gene transcripts and an increase in *Cldn2* expression. Therefore, they reported a switch in E-cadherin localization from the membrane to the cytoplasm of IECs (Cattin et al., 2009).

Using a constitutive intestinal epithelial conditional knockout mouse model, Darsigny *et al.* showed that the loss of HNF4α decreased mucosal ion transport without affecting barrier permeability and this, at a moment preceding the spontaneous manifestation of IBD-related

symptoms. CLDN15 acts as a paracellular ion transporter without interfering with TJ barrier function. A direct correlation between the loss of HNF4α expression and the decrease of *Cldn15* gene transcript and protein levels was also established in this model system. Finally, *CLDN15* was confirmed as being a direct target for HNF4α (Darsigny et al., 2009).

1.5 Premises and hypothesis

HNF4α is an important regulator of cell metabolism, differentiation, and morphogenesis. Recent studies place this TF as a key component in inflammation processes and to be directly linked with the pathophysiology of IBD. The maintenance and integrity of the epithelial barrier is a determining factor for the onset or development of chronic inflammatory diseases. The role of HNF4α in controlling intercellular junctional integrity of the hepatic epithelial physical barrier has been confirmed by several groups. However, little is known about the mechanisms that this NR exerts on the GI tract epithelium in this context. Due to the different nature of knockout models generated, a certain controversy exists as to whether gut-specific deletion of *Hnf4a* directly triggers an increase in intestinal epithelium permeability or not.

Our hypothesis is that the loss of HNF4α in intestinal epithelial cells immediately impacts the function of the epithelial barrier which could make the host more susceptible to bacterial infections. In order to test this hypothesis, we aimed to evaluate the functional impact of a bacterial infection on the intestinal epithelial barrier in mice deleted or not for HNF4α in an inducible manner. To do so, we have generated an intestinal and conditional tamoxifen-inducible *Hnf4a* knockout mouse model and characterized the epithelial barrier associated features (apical complex junctions and mucus) as well as the inflammatory status of this model during infection with an invasive-deficient *Salmonella* Typhimuriun strain.

2. MATERIALS AND METHODS

2.1 Animal model: the conditional tamoxifen-inducible *Hnf4a* knockout mouse model

Hnf4a^{tm1Gonz} mice (*Hnf4a*$^{loxp/loxp}$, catalog no. 004665; Jackson Laboratory) (Hayhurst et al., 2001) were crossed with a mouse transgenic line that expresses the Cre recombinase fused to a mutant form of the human estrogen receptor under the control of the *Villin* gene promoter specific for intestinal epithelial cells (B6.Cg-Tg (Vil1-cre/ERT2) 23Syr/J (Stock No. 020282)) (El Marjou et al., 2004). Mice obtained from these breeding (*Villin*-CreERT2; *Hnf4a*$^{loxp/loxp}$) produce a non-functional and unstable truncated form of HNF4α specifically in the intestinal epithelium. Intraperitoneal injection of tamoxifen results in translocating the Cre-ERT2 fusion protein from IECs cytoplasm to their nucleus. Both male and female *Hnf4a*$^{loxp/loxp}$ (control) and experimental (*Hnf4a*$^{\Delta IEC\text{-}ind}$) adult mice (2 to 4 months in age) were maintained in the animal facility under the recommended guides from the Animal Ethics Committee of the Université de Sherbrooke and the Canadian Council for Animal Care (CCPA).

2.2 Mouse genotyping

Genomic DNA was extracted from mouse toes collected within 7 post-natal days and genotyping was performed by a polymerization chain reaction (PCR). Briefly, each tissue was dissociated in 75 µl of NaOH (25 mM) - EDTA (0.2 mM) solution at 95ºC during 1 h. This solution was neutralized with 75 µl of Tris-HCl (40 mM) at pH 5.5 and centrifuged at 4000 rpm for 3 min in order to sediment remains of the cellular debris. PCRs were then performed with specific primers (see Table 1) to allow the detection of specific modified alleles or transgene associated with the correct genotype. A specific annealing reaction temperature was established for each pair of primers to optimize amplification of each targeted genomic region. PCR products were visualized by migration on 1.5% agarose gels (Roche, Indianapolis, IN, USA) at 110 volts for 30 min.

Table 1: Primers used for PCR genotyping

Genotype	Primers	
CreERT2	*5'-GCGGCATGGTGCAAGTTGAAT-3'*	*5'-CGTTCACCGGCATCAACGTTT-3'*
Hnf4a loxP	*5'-AGAATGACCCTGAAGCACCAGG-3'*	*5'-GCCAGAGGTCTGTGAAACAAGG-3'*

2.3 *In vivo* intestinal permeability assessment

In vivo intestinal permeability (paracellular pathway) was measured in control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice using the FITC-Dextran method (Napolitano et al., 1996). Dextran conjugated to a fluorescent molecule (Fluorescein isothiocyanate) is a non-digestible polymer in the intestine. A solution of 4 kDa FITC-dextran (FD4 Sigma Aldrich, Oakville, Canada) was prepared at a concentration of 80 mg/ml in PBS 1X. Mice were fasted 16 h and then gavaged with this solution at a ratio of 60 mg per 100 g of weight. Both groups of mice were anesthetized after 4 h and cardiac puncture performed. Collected blood was kept for 1 h at room temperature and then centrifuged at 4ºC for 15 min at 3000 g. Serum was collected and FITC-dextran quantified on a spectrophotometer with excitation at 490 nm and emission at 525 nm with reporting the data against a standard curve where the solution stock of FITC-dextran (80 mg/ml) was serially diluted in mouse serum within a range of 500 to 0.97 ng/ml.

2.4 Mouse sacrifice and tissue fixation methods

Mice were sacrificed by cervical dislocation after being anesthetized by an intraperitoneal injection of ketamine-xylazine (10 µl per 10 g of weight). Two methods were used for tissue fixation. First, intestinal tissues (small intestine and colon segments) were placed in cassettes, fixed in fresh 4% PFA and then incubated at 4ºC for approximately 18 h. Subsequently, the cassettes were washed with a 70% ethanol solution and the samples were stored at 4ºC in 70% ethanol for a maximum period of one month. Tissues were embedded in paraffin blocks and then cut into 5 µm histological sections. The second method used the Carnoy fixation, which is necessary for preserving the mucus layers in close proximity to the intestinal epithelium. Cassettes containing ileum and colon segments were placed 2 h at 4ºC in Carnoy's solution (60% ethanol, 30% chloroform, 10% glacial acetic acid). After incubation, the solution was removed and the tissues were transferred in 100% ethanol. Finally, all samples were transferred to the Electron Microscopy & Histology Research core of the FMSS at the Université de Sherbrooke for circulation and inclusion.

2.5 Staining

2.5.1 Hematoxylin & Eosin (H&E)

The slides containing intestinal sections were heated at 60ºC for 15 min and rehydrated with progressive decreasing concentrations of ethanol (see Table 2). Subsequently, the samples

were stained for 3 min with a filtered 0.1% Gil III Hematoxylin solution (Poly Scientific; Cat # s210-8oz) and washed in tap water for 5 min. The ileum and colon sections were placed in a 1% acid alcohol solution (95% HCl/EtOH) for approximately 30 sec and rinsed in running water for 1 min. Next, the slides were incubated for 1 min in water containing ammonia (1 g of sodium bicarbonate in 1 liter of distilled water, pH 8.0) and then washed for 5 min. Once the washing step was completed, the slides were stained with an Eosin solution (Fluka St. Louis, MA, USA) for 1 min and 30 sec and rinsed in running water for 5 min. The dehydration tissue (see Table 3) was performed by 2 min washes in increasing concentrations of ethanol (70%, 95%, and twice 100%), followed by immersion in xylene twice, 1 min each. Finally, samples were covered with a glass slide (VWR, West Chester, PA, USA) using the Vectamount reagent (Vector Laboratories, Burlingame, CA, USA). Analysis of the slides was done using a multi-leaf scanner with visible lights (Nanozoomer 2.0-RS, Hamamatsu, Japan). Images were taken using the NDP.view2 software.

Table 2: Tissue rehydration and dehydration protocol

Tissue rehydration process		**Tissue dehydration process**	
Solution	***Incubation time***	***Solution***	***Incubation time***
Xylene	2x 5 min	EtOH 100%	1x 2 min
EtOH 100%	2x 2 min	EtOH 95%	1x 2 min
EtOH 95%	1x 1 min	EtOH 80%	1x 2 min
EtOH 80%	1x 1 min	EtOH 70%	1x 2 min
EtOH 70%	1x 1 min	Xylene	1x 1 min
H_2O	1x 1 min	Xylene	1x 1 min

2.5.2 Alcian Blue – Periodic acid Schiff (AB-PAS)

Slide sections from tissues previously fixed in PFA or Carnoy's solution were heated for 15 min at 60ºC and then rehydrated as shown in Table 2. Next, the slides were incubated for 3 min in 3% acetic acid pH 2.5 and then stained in a 1% Alcian Blue reagent solution for 15 min at room temperature (RT) in a humid chamber. Tissue sections were washed in running tap water for 1 min. Then, the samples were incubated for 5 min in 0.5% periodic acid, rapidly washed in tap water and again incubated for 10 min in a solution of Schiff's reagent. After being washed for 5 min in running tap water, counterstaining was performed with Gill III Hematoxylin for 45 sec. Finally, the slides were washed in running tap water for 2 min and dehydration and the mounting process was performed as in section 2.5.1.

AB-PAS coloration from paraffin-embedded ileum sections were used to count and measure the size (µm) of goblet cells. At least 20 crypt-to-villus axes in the ileum of control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice were analyzed using Fiji (ImageJ version 2.0.0-rc-69/1.52p). The total number of stained goblet cells was expressed per crypt or villus units.

2.6 *Salmonella* Typhimurium infection

Salmonella enterica serovar Typhimurium strain SB103 (*invA :: strep*) was seeded in 10 ml of Luria Bertani (LB) medium (BioShop, Canada) containing 100 mg/ml of streptomycin antibiotic. The bacterial culture was grown overnight at 37ºC with shaking at 210 rpm. One ml of this culture was then transferred to an eppendorf and centrifuged for 4 min at 6000 rpm. The pellet was then resuspended in 10 ml of PBS 1X. Mice were force-fed with a 100 µl aliquot of this resuspension that contained approximately 10^7 colony forming units (CFU). Mice were monitored daily and sacrificed 4 days after infection. The number of bacteria was determined in the liver, spleen and feces. To accomplish this, a fragment of each tissue was removed, weighed, and then homogenized in 1 ml of PBS 1X and serial dilutions were seeded on LB agar containing streptomycin. Total RNA and protein samples were also prepared from collected ileum and colon biopsies.

2.7 RNA isolation

Approximately 1 cm of small intestine and colon tissues were put in 750 µl of RNA later solution (QIagen, Toronto, Canada) and kept at -80ºC until further use. The tissues were then removed and disrupted mechanically (5 mm stainless steel beads) in 750 µl of denaturation solution (RLT/β-mercaptoethanol buffer) from the RNeasy extraction kit (QIagen, Toronto, Canada). Samples were placed in a TissueLyser LT (QIagen, Toronto, Canada) for 3 min at 50 Hz amplitude, then incubated 10 min on ice. A second round of disruption was performed for 2 min at 50 Hz amplitude, followed by 10 min of incubation on ice. Samples were then centrifuged at 4ºC for 15 min at 13 000 rpm. Supernatants were collected and kept at -80ºC until further use. To further isolate total RNA, 300 µl of supernatant were mixed with 300 µl of 70% ethanol. The mix was transferred to a RNeasy Mini spin column and centrifuged for 15 sec at ≥ 8000 g. Each column was subjected to successive washing steps, adding 700 µl of RW1 buffer or RPE buffer. Finally, each column was placed into a new collection tube

and 40 µl RNase-free water was added directly to the spin column membrane and centrifuged for 1 min at ≥ 8000 g to elute the RNA.

RNA concentration was determined by measuring samples absorbance at 260 nm in a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, USA). RNA purity was determined by measuring A260/A280 nm and A260/A230 nm ratios. In addition, RNA integrity was visualized on 1% agarose gels with the use of a molecular weight standard.

2.8 Reverse transcription

Samples were prepared with 1 µg of RNA, 1 µl of 50 mM EDTA and water to complete the final volume to 10 µl. A master mix was made with 4 µl of 5X SSIV buffer (Invitrogen, Burlington, Canada), 1 µl of DTT (100 mM), 0.8 µl of dNTPs (25 mM) (Amersham Biosciences, Laval, Canada), 2.4 µl of Oligos p(d)T (Amersham Biosciences, Laval, Canada), 0.6 µl of RNase inhibitor (Roche, Laval, Canada) and 1.7 µl of a 1:10 dilution of the RT Superscript reverse transcriptase (Invitrogen, Burlington, Canada). Samples were heated at 70ºC for 5 min, and then placed on ice for another 5 min. 10 µl of the master mix was added to 10 µl of the sample and this 20 µl was further incubated at 50ºC for 1 h. The reaction was stopped by incubation at 95ºC for 5 min. For 1 µg of cDNA, 180 µl of water were added and stored at -20ºC until further usage.

2.9 Quantitative real-time RT-qPCR

Quantitative PCR (qPCR) was performed with 2 µl of cDNA combined to 18 µl of master mix made with 10 µl of FastStart Essential DNA Green Master (Roche, Laval, Canada), 6 µl of water and 1 µl of each primer (IDT Technology, Coralville, USA). The primer sequences used for each reaction were designed to obtain amplicons around 100 bp and are found in Table 3. The qPCR was carried out in an SYBR Green fluorescence temperature cycler (LightCycler, Roche, Laval, Canada) with denaturation cycles at 95ºC for 10 sec, annealing cycles at 60ºC for 10 sec, and extension cycles at 72ºC for 20 sec. Each sample was amplified in duplicate and the standard curve in triplicate, where relative expression of target genes was calculated by comparison with the expression of the housekeeping gene *TBP* (Tata-box binding protein).

Formula: $\text{Efficacy}_{\text{target gene}}^{(\text{CqCalibrator} - \text{CqSample})} \text{ X } \text{Efficacy}_{\text{reference gene}}^{(\text{CqSample} - \text{CqCalibrator})}$

Table 3: Real-Time RT-qPCR primers (Mus musculus)

GENES	FORWARD	REVERSE
RegIIIγ	*5'-TGCATTGTG ACAAAAATACCC-3'*	*5'-CAGGTATGGGTGCAGTTTGA-3'*
RegIIIβ	*5'-AGGTAAAGCAGGTCGGCTAA-3'*	*5'-AGCATTTGGCACAGACTCAA-3'*
Ang4	*5'-TCTCCAGGA GCACACAGCTA-3'*	*5'-TGAGCCAGAGTTGGAGGAAT-3'*
Lyz	*5'-CCAGAACTCTGAAAAGGAATGG-3'*	*5'-CCAGTATCGGCTATTGATCTGA-3'*
Defa20	*5'-ACCAGGCTGTGTCTGTCTCC-3'*	*5'-GGCAGCAGAACAAAAGTCGT-3'*
Defa5	*5'-TATCTCCTTTGGAGGCCAAG-3'*	*5'-CAGCTGCAGCAGAATACGAA-3'*
Defa3	*5'-CCAGGCTGATCCTATCCAAA-3'*	*5'-GTCCCATTCATGCGTTCTCT-3'*
$SPLA_2$	*5'-CACCACTCCACTGCCTTGAAT-3'*	*5'-GCAGGAAGTTGGATGCCAA-3'*
Tlr4	*5'-TCAGAACTTCAGTGGCTGGA-3'*	*5'-CCTGGGGAAAAACTCTGGAT-3'*
Myd88	*5'-GCGACTATACCAACCCTTGC-3'*	*5'-TGTAGACAGGACGGCATCAG-3'*
Nlrp3	*5'-AACCTGGGCAACAATGATCT-3'*	*5'-TCTTCCTGGAGCGCTTCTAA-3'*
JAM-A	*5'-CTTGATTTTTGGCGTCTGGT-3'*	*5'-GGAACGACGAGGTCTGTTTG-3'*
ZO-1	*5'-TATCCCACAAGGAGCCATTC-3'*	*5'-GGCTCCAACAAGGTAATTCG-3'*
Ocln	*5'-GAGTACAGTGCTGCTGCTGAT-3'*	*5'-TCCCACCATCCTCTTGATGT-3'*
Cldn3	*5'-GCACCCACCAAGATCCTCTA-3'*	*5'-GTCCTCTTCCAGCCTAGCAA-3'*
Cldn1	*5'-TTAGTGGCCACAGCATGGTA-3'*	*5'-GGGACAGGAGCAGGAAAGTA-3'*
Cldn2	*5'-TCCAGAGCTCTTCGAAAGGA-3'*	*5'-TTCTTGGATCCGAGCCTCTA-3'*
Cldn4	*5'-AACCCTTCCGTTGATTAGCA-3'*	*5'-CACTGGGCTGCTTCTAGGTC-3'*
Cldn7	*5'-TGTTCCTGGATTGGTCATCA-3'*	*5'-CCTGGACAGGAGCAAGAGAG-3'*
Cldn15	*5'-TTCCTGGGCCTCTTTCTAGG-3'*	*5'-AGCAGGTGGAGAAGACACAGAT-3'*
Muc2	*5'-CTTCAATTGGTCCTGTCCAT-3'*	*5'-CCTGCTTGGGAGGATCAAAA-3'*
Muc1	*5'-TACCAAGCGTAGCCCCTATG-3'*	*5'-AGTGACCACTGAGGGAGCAG-3'*
Muc3	*5'-CTTCCAGCCTTCCCTAAACC-3'*	*5'-TGGCTAAACACGCTTCTCCT-3'*

Tff3	*5'-GCTGCCATGGAGACCAGA-3'*	*5'-GAGCCTGGACAGCTTCAAAA-3'*
Retlnb	*5'-CGCAATGCTCCTTTGAGTCT-3'*	*5'-CCACAAGCACATCCAGTGAC-3'*
Hnf4α	*5'-GGTCAAGCTACGAGGACAGC-3'*	*5'-ATGTAGTTGGCCCACTCGAC-3'*
IL-18	*5'-AGTAAGAGGACTGGCTGTGACC-3'*	*5'-TCATCTTCCTTTTGGCAAGC-3'*
IL-1β	*5'-TGTGTAATGAAAGACGGCACA-3'*	*5'-TACCAGTTGGGGAACTCTGC-3'*
GsdmD	*5'-GCTGCTAGCTAAGGCTCTGG-3'*	*5'-GGATTCTTTTCATCCCAGCA-3'*
Casp1	*5'-CTGTCAGGGGCTCACTTTTC-3'*	*5'-TCAGCAGTGGGCATCTGTAG-3'*
TNFα	*5'-GATTATGGCTCAGGGTCCAA-3'*	*5'-CATTCGAGGCTCCAGTGAAT-3'*
Cdh1	*5'-CCTGCCAATCCTGATGAAAT-3'*	*5'-GTCCTGATCCGACTCAGAGG-3'*
TBP	*5'-GGGGAGCTGTGATGTGAAGT-3'*	*5'-GGAGAACAATTCTGGGTTTGA-3'*
Agr2	*5'-ACCGGCTCTACGCTTATGAA-3'*	*5'-TCTGCAAGTCCACAGTGCTT-3'*
Klf4	*5'-CAGTATACATTCCGCCACAGC-3'*	*5'-GTCTGGGCTTCCTTTGCTAAC-3'*
Fut2	*5'-GAGTCAAGGGGAGGGAGAAC-3'*	*5'-CCAGGGCTACAGAAGTGGAC-3'*
Fcgbp	*5'-TGGTTCTCAGGGGAAGACAC-3'*	*5'-ACACAGGGCATCTTCCAATC-3'*
Spdef	*5'-GCCTGGATGAAGGAGAGGAC-3'*	*5'-GGCTTGAGCAGCAGTTCTTT-3'*
Math1	*5'-CGATGATGGCACAGAAGGA-3'*	*5'-GGGGAAAACTCTCCGTCACT-3'*

2.10 Immunofluorescence

Indirect immunofluorescences were performed on sections of intestinal tissues previously fixed in PFA or Carnoy solution. To deparaffinized the samples, the slides containing the tissue sections were exposed to 60ºC for 15 min. Subsequently, a tissue rehydration process was performed (see Table 2) and antigens recovery was carried out using 10 mM citrate buffer, pH 6 during 12 min in a microwave (Fisher Scientific, Canada). The samples were then cooled and washed in running water for 20 min and tissue blocking was performed using a PBT solution (PBS 1X, BSA 2%, Triton X100 0.2%). After blocking for 40 min at room temperature (RT), the samples were incubated in a humid chamber at 4ºC overnight, with primary antibodies (Ab) diluted in PBT (Table 4). Next, the slides were washed three times for 10 min in PBS 1X and immediately incubated with the secondary Ab for 1 h in a humid

chamber at RT with light protection. After that, the slides were washed twice in PBS 1X and stained for 30 sec (protected from light) with a solution of 4, 6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, St-Louis, MA, USA) diluted 1: 100 in PBS 1X to stain the cell nucleus or with an Alcian Blue solution. After the counterstaining was completed, the samples were washed 3 times for 5 min in PBS 1X and then quickly rinsed in distilled water. Finally, the slides were mounted with the Immu-Mount mounting medium (Fisher Scientific, Canada) and observed at 20x (HC PLAN APO 20X/na=0.7/WD=0.59mm) and 40x (HCX PL APO 40X/na=0.85/WD=0.24) under the microscope Leica DM 2500 OPTIGRID (Leica Microsystems CMS GmbH, Germany) using Metamorph software or at 20x (HC PL FLUOTAR 20X/na=0.50/WD=1.15mm) under the microscope Leica DMLB2 (Leica, Germany) using Leica FireCAM 3.4.1 software.

Table 4: Primary and secondary antibodies used for IF

Primary antibodies	Conditions	Source	Cat. Number	Provider
MUC2	1:200	Rabbit	Ab133555	abcam
UEA-1	1:750	FITC-coupled	L9006	Sigma
Secondary antibodies				
IgG Fab'2 (Alexa 488) (anti-rabbit)	1:300	Goat	4408S	Cell Signaling

2.11 Fluorescence in situ hybridization (FISH)

Ileum and colon sections of *Hnf4a*$^{\Delta IEC\text{-}ind}$ and control mice that were fixed in Carnoy and embedded in paraffin were deparaffinized and hydrated with distilled water as shown in Table 2. Sections were then hybridized overnight at 50°C with 10 ng/µl of a general bacterial 16S rRNA probe (EUB338) (IDT Technology, Coralville, USA). Both the positive probe and a negative binding control (nonsense probe (NS_EUB338)) were prepared in the hybridization buffer (20 mM Tris-HCl, 0.9 M NaCl, 0.1% SDS, pH 7.4) (Table 5). DAPI was used to stain cell nucleus for 30 sec. Slides were then washed twice with wash buffer (20 mM Tris HCl, 0.9 M NaCl, pH 7.4) and sections mounted with Immu-Mount mounting medium (Fisher Scientific, Canada). Images were obtained at 20x (HC PLAN APO

20X/na=0.7/WD=0.59mm) and 40x (HCX PL APO 40X/na=0.85/WD=0.24) with a Leica DM 2500 OPTIGRID microscope (Leica Microsystems CMS GmbH, Germany).

Table 5: Fish probes primer sequences

PRIMER	SEQUENCE
CY3_EUB338_SENSE	*5'-/5Cy3/GCT GCC TCC CGT AGG AGT-3'*
CY3_NS_EUB338	*5'-/5Cy3/CGA CGG AGG GCA TCC TCA-3'*

2.12 Electron microscopy analysis

Intestinal tissues from 2- to 4-month-old *Hnf4a*$^{AIEC\text{-}ind}$ and control littermates were fixed for a minimum of 12 h with 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 at 4ºC. Samples were then transferred to the Electron Microscopy & Histology Research core of the FMSS at the Université de Sherbrooke for further processing. Briefly, samples were rinsed twice with 0.1 M cacodylate buffer pH 7.4 and incubated for 90 min in 1% osmium tetroxide solution in cacodylate buffer. Sample dehydration was then performed using increasing concentrations of ethanol (70, 85, 95 and 100%) and propylene oxide. Then, tissues were infiltrated in Epon 812 resin which was left at 60ºC for 48 h to polymerize. Sections (80 nm) were ultra-stained using a Leica Ultracut UCT ultramicrotome (Leica MicroSystem, Concorde, On, Canada) in lead citrate (7 min) and uranyl acetate (30 min). The images were observed under a Hitachi H-7500 transmission electron microscope (Hitachi, Schaumburg, IL, USA).

2.13 Extraction and quantification of proteins from animal tissue

Approximately 1 cm of ileum and colon tissues were put in 750 µl of RIPA lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.5% Triton X100, 1 mM EDTA, 0.2% SDS, and 0.5% sodium deoxycholate), to which was added a cocktail of protease and phosphatase inhibitors (2% of PIC (Sigma Aldrich, Oakville, Canada), 0.4 mM Na3PO4 (Sigma Aldrich , Oakville, Canada), 50 mM NaF (Sigma Aldrich, Oakville, Canada)) and then disrupted mechanically using 5 mm stainless steel beads (QIagen, Toronto, Canada) in a TissueLyser LT (QIagen, Toronto, Canada). Samples were homogenized for 3 min at 50 Hz amplitude and incubated 10 min on ice. A second round of disruption was performed for 2 min at 50 Hz amplitude, followed by 10 min of incubation in ice. Next, the samples were centrifuged at 4°C for 15 min at 13,000 rpm and the supernatants were collected and frozen

at -80°C. The protein extracts were quantified by a Bicinchoninic Acid (BCA) assay (Life Technologies inc., Burlington, Canada). BCA working reagent (WR) was prepared by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B. A standard curve with Bovine Serum Albumin (BSA) (Roche, Laval, Canada) was prepared with BSA stock (1 mg/ml, dissolved in H_2O) and then, 8 serial dilutions were made to cover a range of 25 to 1 µg/µl. 25 µl of each sample (2 µl of sample, 23 µl of water) in 200 µl of the WR was added to a 96 wells plate as duplicate. The plate was incubated at 37°C for 30 min (Isotemp Incubator, Fisher Scientific, Canada). Samples concentrations were determined by reading the absorbance at 562 nm on a spectrophotometer (Versamax, Molecular devices).

2.14 IgA detection in the intestinal epithelium

A mouse IgA ELISA kit was used for the detection of intestinal epithelial IgA (code 3865-1AD-6, Mabtech, Sweeden). High binding ELISA plates (Corning, USA) were coated and incubated overnight at 4ºC, with 100 µl/well of the monoclonal Ab MT45A diluted at 2 µg/ml in 1x PBS pH 7.4. After 3 washes with 1x PBS - 0.05% Tween-20 (PBS-T), the plates were incubated for 1 h at RT with the blocking solution (1x PBS, 0.05% Tween-20 and 1% BSA). Serial 1:2 dilutions of the standard mouse IgA were prepared in the incubation buffer to cover a concentration range between 500 ng/ml and 0.97 ng/ml. Following 3 washes with PBS-T, 100 µl/well of the standard dilutions or samples (1: 100 for ileum and 1:50 for colon) were added to the plates and incubated for 2 h at RT. The plates were then washed and incubated with 100 µl/well of the secondary antibody (MT39A-ALP) conjugated to alkaline phosphatase (diluted 1:1000) for 45 min at RT. The reaction detection was done by adding 100 µl/well of p-nitrophenyl-phosphate (pNPP) in 1M diethanolamine buffer with 1 mM $MgCl_2$ and stopped by adding 50 µl/well of 3M NaOH. The plates were read at 405 nm in a spectrophotometer (model Flextation 3).

2.15 Statistical analyses

Statistical significance was calculated with parametric and non-parametric test using GraphPad Prism Software Version 8. Unpaired t-test and Mann-Whitney test was used to compare two experimental groups and Anova test for multiple comparisons, was applied to compare experimental groups. Statistical significance is presented as *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$. All data are expressed as the mean ± SEM.

3. RESULTS

3.1 Increased paracellular permeability of the intestinal mucosa in the absence of epithelial HNF4α.

To study the putative role of HNF4α in the maintenance of the intestinal barrier, we decided to disrupt this gene during mouse adulthood using an inducible conditional knockout strategy. Deletion of *Hnf4a* was generated from the crossing of mice that contained loxP sites surrounding exons 4 and 5 of the *Hnf4a* gene (*Hnf4a*$^{loxP/loxP}$) with the transgenic mouse model *Villin*-CreERT2 that produces inducible CreER fusion recombinase specifically in the intestinal epithelium. Activation of this CreER depends on its binding to tamoxifen (TAM) allowing its translocation from the cytoplasm to the nucleus in order to be able to recombine available loxP sites.

During 5 consecutive days, we performed intraperitoneal injections with TAM (1 mg/ 100 µl ethanol/corn oil, dilution 1:9) in both control (*Hnf4a*$^{loxP/loxP}$) and mutant (*Villin*-CreERT2; *Hnf4a*$^{loxp/loxp}$ or *Hnf4a*$^{\Delta IEC\text{-}ind}$) followed by a 3 days post-treatment period to allow for the complete renewal of the intestinal epithelium and deletion fixation before sacrifice. *Hnf4a* was quantified by qPCR in different segments of the GI tract. As seen in Fig.5A, *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice showed a significant and robust reduction in relative *Hnf4a* mRNA expression when compared to control mice, regardless of the investigated intestinal section (small intestine $p=0.0079$ and colon $p=0.0033$). In addition, inducible deletion efficiency in our experimental model was near 100%.

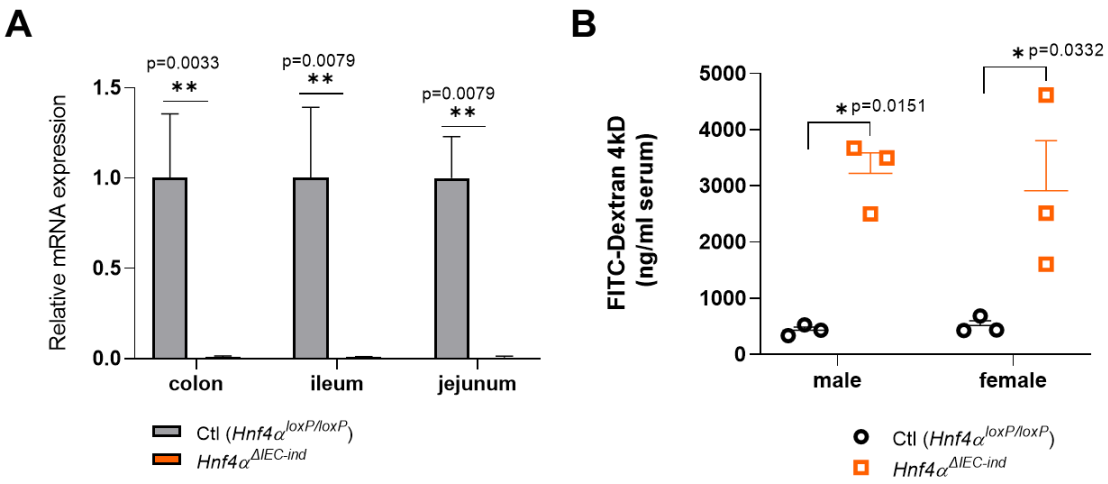


Fig. 5: Intestinal permeability is increased in the absence of HNF4α. A) qPCR quantification of *Hnf4a* mRNA levels from various intestinal sections. mRNA levels are expressed relative to the amount detected in control mice and normalized to *TBP* housekeeping gene transcripts. B) *In vivo* paracellular permeability analysis was done by measuring FITC-dextran 4kD in serum samples from control ($Hnf4a^{loxP/loxP}$) and $Hnf4a^{\Delta IEC\text{-}ind}$ mice. Animals were gavaged 4 h before sacrifice. Concentration levels are shown as mean values (± SEM) relative to controls. n=3 mice per group in a single experiment. Each data point corresponds to the average of the values obtained in duplicate for each animal on the same plate. For the first graph an *unpaired t-test* or *Mann-Whitney* test was used to measure significance and for the second graph was applied a *two-way Anova* test.

To determine if the removal of HNF4α can generate immediate changes in intestinal barrier functionality, an *in vivo* paracellular permeability test was performed. Mice were gavaged with the FITC-dextran (4 kDa) probe, a glucose polymer coupled to fluorescein isothiocyanate marker that does not normally cross the mucosa when the barrier is intact (Woting and Blaut, 2018; Wang et al., 2015). Serum from mice was extracted 4 h post-administration and fluorescence levels determined from extrapolation against a standard curve obtained with known concentrations of FITC-dextran. As indicated in Fig. 5B, both male (7.5 times, p=0.0151) and female (5.6 times, p=0.0332) $Hnf4a^{\Delta IEC\text{-}ind}$ mice exhibited high accumulation of the probe in their serum, reaching mean values around 3,225 ng/ml (males) and 2,914 ng/ml (females) when compared to control mice of both sexes (males: 432 ng/ml; females: 516 ng/ml). This result indicates that the loss of HNF4α in the intestinal epithelium leads to weaker intestinal epithelial barrier integrity.

3.2 Loss of HNF4α does not influence infection outcomes from attenuated *Salmonella* Typhimurium strain SB103

Taking into account the permeability results obtained with our experimental mouse model, we carried out biological infections of these mice to verify if defects observed in epithelial integrity can lead to greater accessibility of luminal bacteria into the intestinal mucosa. For this, we used *Salmonella enterica* serovar Typhimurium, one of the most studied Gram (-) bacteria and commonly isolated during infections of the GI tract (Cheng et al., 2019; Rabsch et al., 2001). *Salmonella* is able to invade the epithelium by internalization from Peyer's patches via M cell or by direct interaction with the brush border of IECs (Lhocine et al., 2015).

Salmonella Typhimurium is capable of inducing a systemic disease and, in mice with a C57BL/6 genetic background, lead to death after 5-7 days of infection due to their high genetic susceptibility (Brown et al., 2013). Therefore, to specifically investigate the paracellular pathway as the main entry for such bacteria, we chose a derivative of the wild strain SL1344 (*Salmonella* Typhimurium SB103 *invA*::strep), which has a genetic mutation in the *invA* gene encoded within highly conserved *Salmonella* pathogenicity islands (SPI-1), thus limiting bacteria invasiveness through IECs, but not overall virulence (Galan and Curtiss, 1991).

Control and $Hnf4a^{\Delta IEC\text{-}ind}$ mice were challenged orally with 10^7 colony forming units (CFU) of this bacterial strain. Infection was monitored at different time points by evaluating changes in animal bodyweight and bacterial CFU from feces. Mice were then sacrificed 4 days post-infection (pi) while colon and ileum samples were collected for histopathological analysis, as well as liver and spleen tissue for characterization of the infection at the systemic level.

As seen in Fig. 6A, $Hnf4a^{\Delta IEC\text{-}ind}$ mice showed a significant decrease in the relative levels of *Hnf4a* gene transcripts in both the ileum (males: p=0.0003, females: p=0.0234) and colon (males: p=0.0012, females: p=0.0403) approximately 7 days after TAM treatment. CFU counts from stools homogenates of $Hnf4a^{\Delta IEC\text{-}ind}$ male (Fig. 6B) and female (Fig. 6C) mice showed very similar levels of bacterial burden when compared with the CFU obtained for their respective controls.

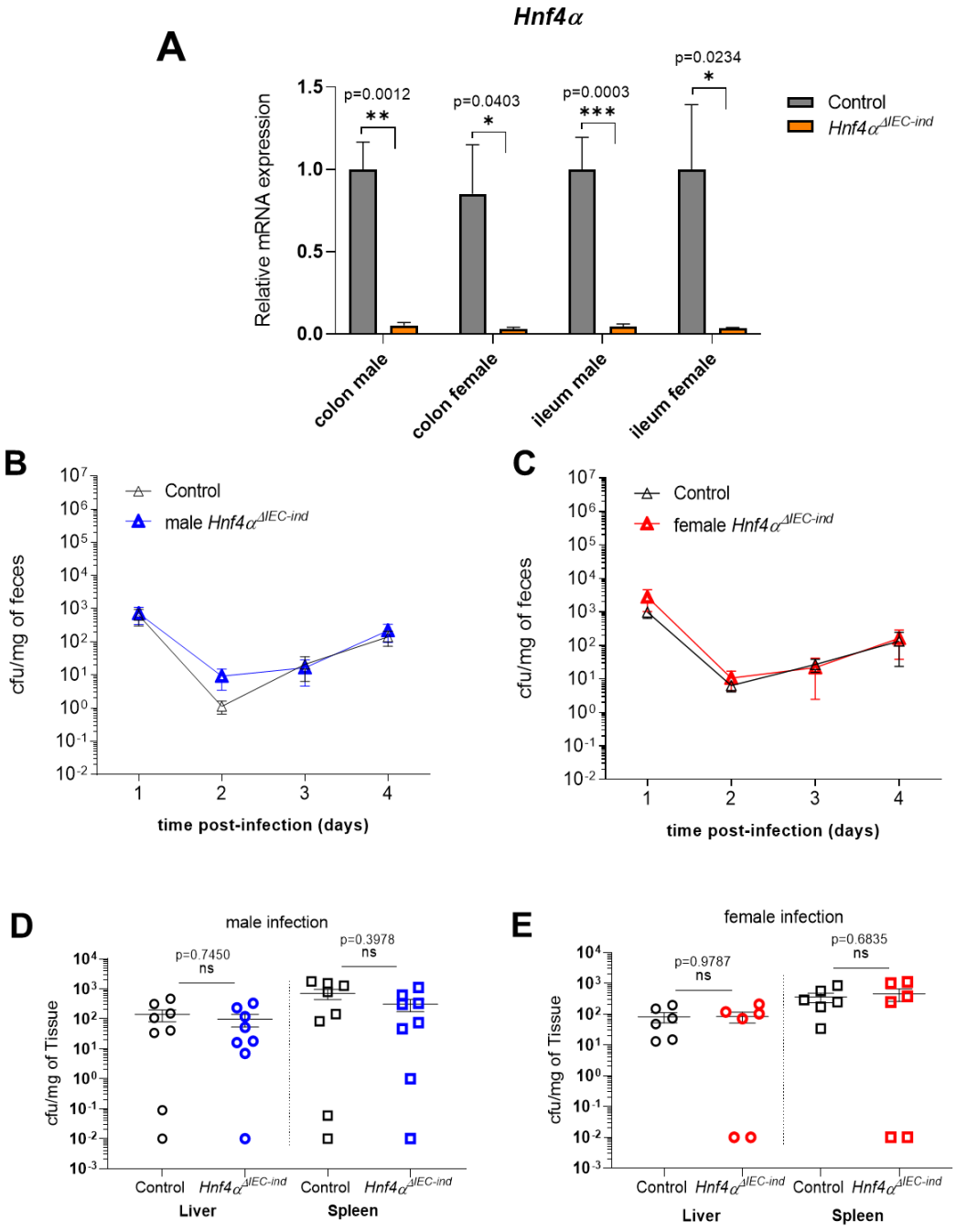


Fig. 6: Loss of HNF4α does not lead to increased susceptibility to *Salmonella* infection. Control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice were orally infected with the attenuated *Salmonella* Typhimurium strain SB103. A) Total RNA was isolated from the ileum and colon of control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice (n = 6-8 per group) and qPCR was performed to quantify *Hnf4a* gene transcripts. Bacterial counts were done in feces during the 4 days of the infection test (B: males and C: females), liver and spleen tissues (males: D and E: females). For each graph, an *unpaired t-test* or non-parametric *Mann-Whitney* test was used to measure significance.

Interestingly, the analysis of the CFU in stool at 48 h pi showed a significant reduction in the concentration of *Salmonella* when compared to the load observed at 24 h pi in each treated group (Fig. 6B and C). This effect was more pronounced in the control male mice (550-fold less infected compared to D1 pi of the same group, p=0.0002), but without being significant compared to D2 pi of the *Hnf4α*$^{\Delta IEC\text{-}ind}$ male mice (8.1-fold, p=0.1754) (Table 3, Annexes). However, the infection was more prominent and relatively equivalent in the different groups evaluated at day 3 and day 4 pi.

In the case of systemic spread of *Salmonella* Typhimurium SB103, we did not detect considerable variation between the number of CFU for both *Hnf4a*$^{\Delta IEC\text{-}ind}$ and control mice (males: Fig. 6D, females: Fig. 6E), with recorded average values between 10^1 and 10^3 CFU/mg of tissue. Nevertheless, this experiment revealed a tendency towards lighter colonization in *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice (average of 97 CFU/mg of liver tissue and 312 CFU/mg of spleen tissue) when compared to controls (140 CFU/mg of liver tissue and the 700 CFU/mg of spleen tissue). Finally, the different infected groups did not show remarkable weight variations during the 4 days of infection (data not shown).

3.3 The apical junction complex appears to be modified in HNF4α$^{\Delta IEC\text{-}ind}$ mice

The flow of molecules passing through IECs is regulated by an apical complex of junctions composed of TJ, AJ, and desmosomes. These junctions are directly involved in epithelial barrier functionality in order to protect against the passage of possible pathogens (Odenwald and Turner, 2017).

Given that the permeability was increased in the intestinal mucosa in our mutant mice, we decided to visualize the contacts established between the plasma membranes of the IEC monolayer by electron microscopy in infected conditions (Fig. 7).

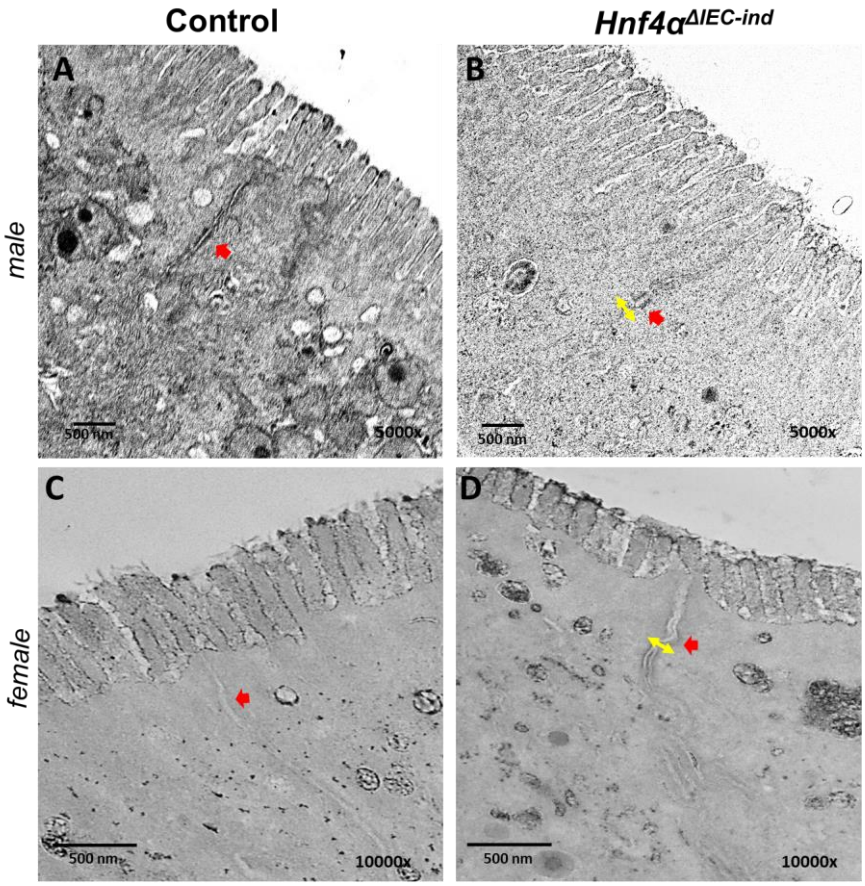


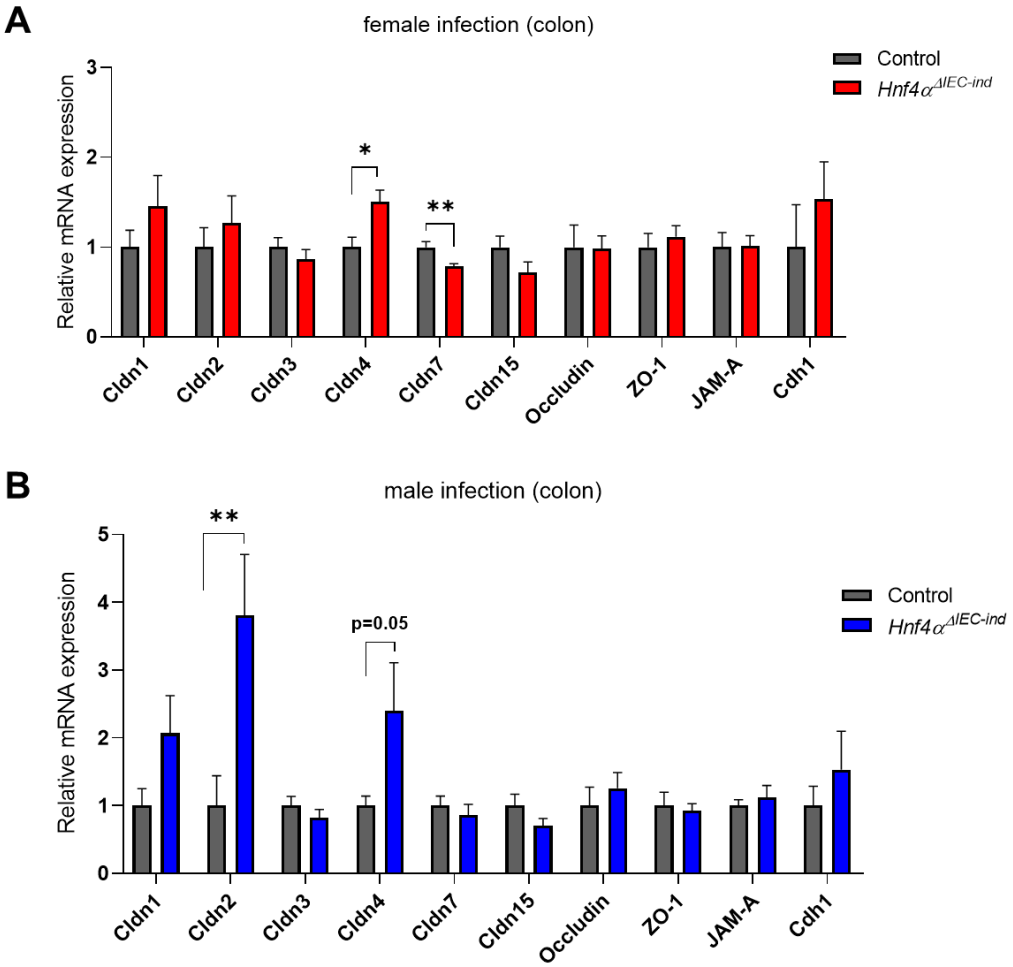
Fig. 7: Distension of the apical complex junctions in the absence of HNF4α. Electron microscopy images showing the intercellular space within apical complex junctions of control (A: male and C: female) and *Hnf4α*$^{\Delta IEC\text{-}ind}$ mice (B: male and D: female). Magnification = 10,000 X (n=2 per group, about 5 cells analyzed per animal). Yellow arrows indicate the distention in the TJ.

In comparison to control animals, both male and female *Hnf4α*$^{\Delta IEC\text{-}ind}$ mice showed an increase in the intercellular space of TJ, as shown in Fig. 7B and 7D (red and yellow arrows).

3.4 HNF4α intestinal epithelial deletion alters the expression of different gene transcripts related to the protection and integrity of the epithelial barrier after *Salmonella* infection.

3.4.1 Apical complex junctions

Next, we focused our study on gene products that make up the physical barrier of the intestinal epithelium by analyzing the relative levels of their mRNA expression by qPCR. We focused mostly on genes that code for the TJ proteins, since they constitute an important regulator of paracellular permeability. TJ are mainly composed of claudin proteins, which are responsible for the maintenance and sealing of the intercellular space, as well as for the correct cellular polarization from the association with other TJ proteins such as Ocldn and ZO-1 to -3 (Lee et al., 2018).



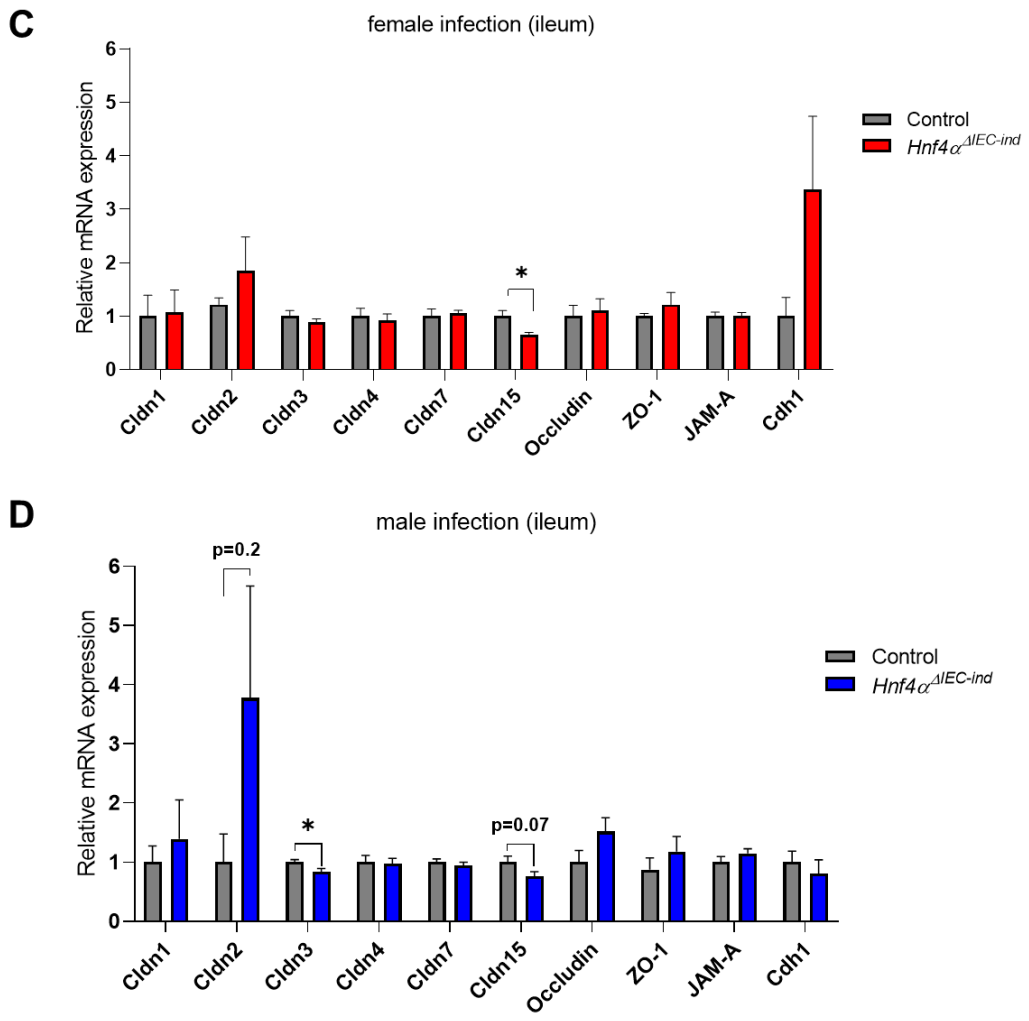


Fig. 8: Expression of cell junction-associated components in the intestine of mice deleted for *Hnf4a*. The mRNA expression levels of different junctional complex components were analyzed by qPCR in female colon (A), male colon (B), female ileum (C) and male ileum (D) of control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice. Expression levels are expressed as mean values (± SEM) relative to controls (n=6-8 mice per group) and were normalized with *TBP* housekeeping gene transcripts. For each graph, an *unpaired t-test* or *Mann-Whitney* test was used to measure significance.

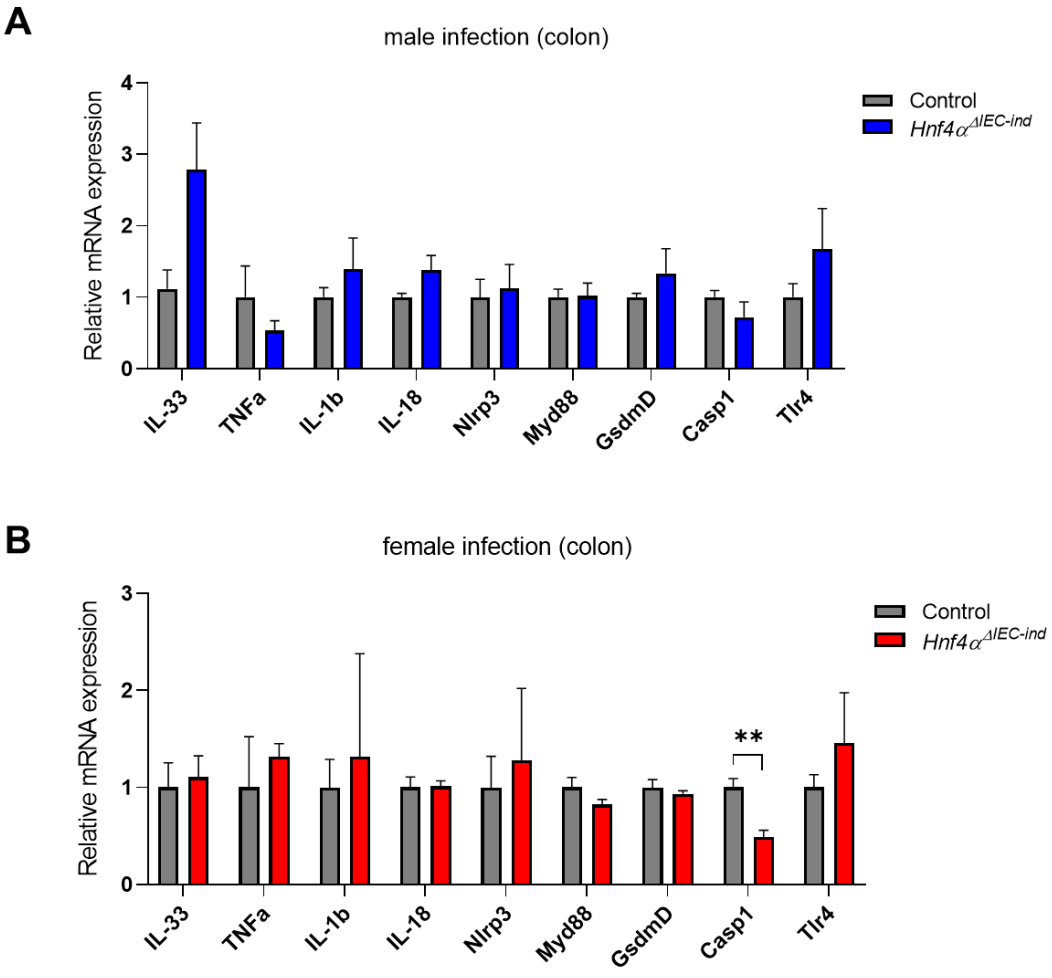
We observed a significant increase in the levels of transcripts associated with the *Cldn4* gene (1.5-fold, p=0.0153) and a significant reduction in the expression of *Cldn7* (1.3-fold, p=0.0043) in the colon of *Hnf4a*$^{\Delta IEC\text{-}ind}$ female mice when compared to their controls (Fig. 8A). *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice showed a significant induction of *Cldn2* (3.8-fold, p=0.0059) and *Cldn4* (2.4-fold, p=0.05) gene transcripts in their colon when compared to controls (Fig. 8B).

On the other hand, while the analysis in the ileum of *Hnf4a*$^{\Delta IEC\text{-}ind}$ female mice displayed a significant downregulation for *Cldn15* gene transcripts (1.3-fold, p=0.0231) (Fig. 8C), only

a tendency towards a lower expression of this gene (p=0.0945) was observed in the ileum of *Hnf4a*$^{\Delta IEC\text{-}ind}$ males (Fig. 8D). Furthermore, *Hnf4a*$^{\Delta IEC\text{-}ind}$ males exhibited a significant reduction of *Cldn3* gene transcripts (1.2-fold, p=0.0438) when compared to their respective controls (Fig. 8D).

3.4.2 Cytokines and immune system modulators

Invasion of epithelial tissues and recognition of conserved motifs in *Salmonella* (LPS, flagellin, etc.) by family members of pattern recognition receptors (PRR) (Toll-like receptors and Nod-like receptors), constitute a sufficient stimulus for the activation of pro-inflammatory signaling cascades (Zha et al., 2019).



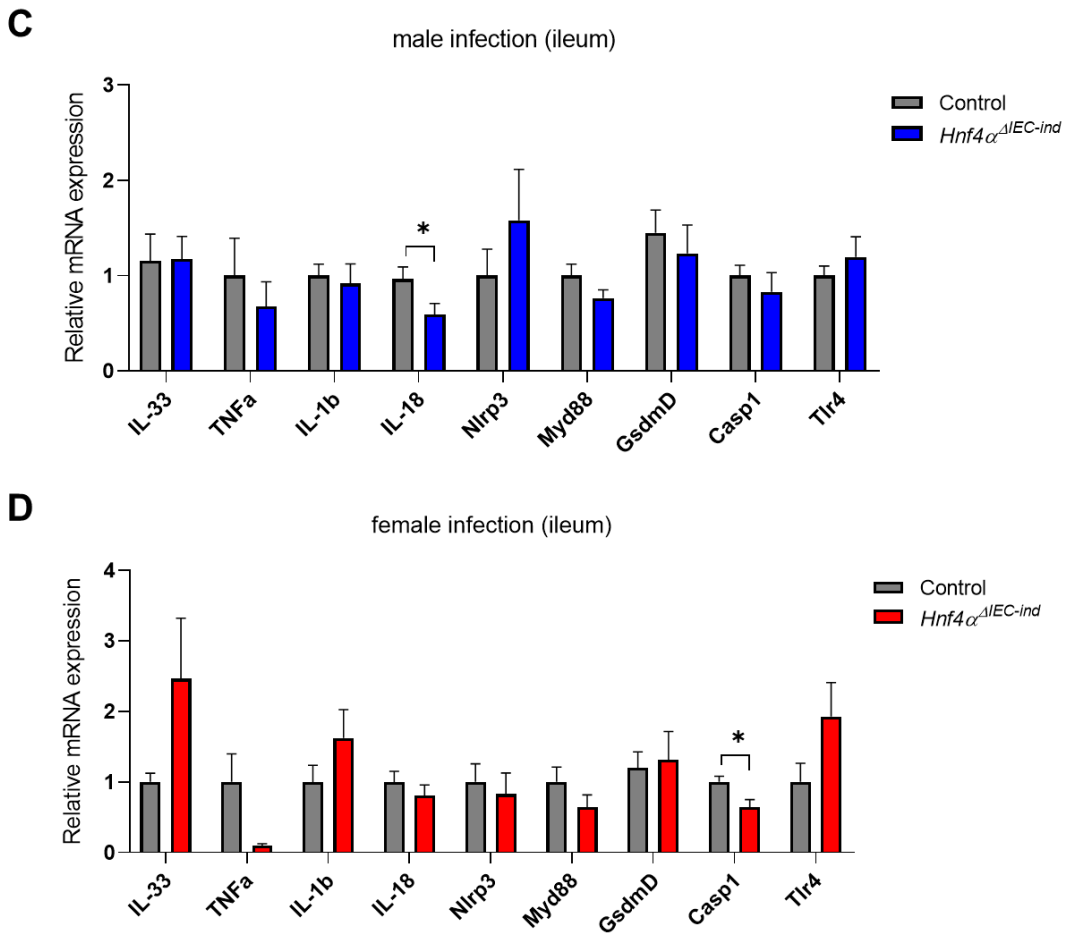


Fig. 9: Expression of immune response modulators from the intestinal epithelium of control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ after infection with the attenuated *Salmonella typhimurium* strain SB103. Relative mRNA expression was quantified by qPCR in male colon (A), female colon (B), male ileum (C) and female ileum (D) of control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice. Expression levels are expressed as mean values (± SEM) relative to controls (n=6-8 mice per group) and were normalized with *TBP* housekeeping gene transcripts. For each graph, an *unpaired t-test* or *Mann-Whitney* test was used to measure significance.

We thus investigated by qPCR the expression profile of a set of genes involved in the inflammatory response including *IL-33*, *IL-18*, *IL-1β*, *Casp1*, *TNFα*, *Myd88*, *Nlrp3*, *GsdmD*, *Tlr4* (Fig. 9). In general, few variations were observed among control and *Hnf4α*$^{\Delta IEC\text{-}ind}$ mice after 4 days of attenuated *Salmonella* infection. Although no significant change was observed in the colon of *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice (Fig. 9A), a significant decrease in the relative levels of *IL-18* mRNA (1.6-fold, p=0.0289) was observed in the ileum of these mice when compared to controls (Fig. 9C).

Hnf4a$^{\Delta IEC\text{-}ind}$ female mice presented a significant reduction in the expression of *Casp1* transcripts in the colon (2.1-fold, p=0.0016; Fig. 9B) as well as in the ileum (1.5-fold, p=0.0250; Fig. 9D) when compared to controls.

3.4.3 Antimicrobial peptides

As mentioned in previous chapters, antimicrobial peptides or AMP, constitute physiological elements that play an essential role in the maintenance of intestinal homeostasis. AMP have a broad spectrum of activity against bacteria, protozoa, and fungi. These peptides or proteins are secreted by Paneth cells and concentrated in mucus, thus controlling the proliferation of the commensal microbiota and the invasion of pathogens (Dupont et al., 2015; Mukherjee and Hooper, 2015). We performed a qPCR analysis to determine if bacterial infection impacted the expression of AMP genes in absence of intestinal epithelial HNF4α. We focused on the ileum since this segment houses the highest number of Paneth cells (Gassler, 2017) and has also been described as the main entry point of *Salmonella* Typhimurium in the intestinal epithelium (Garner et al., 2009; Meyerholz et al., 2002).

Interestingly, induction of both *Defa5* (1.7-fold, p=0.0444) and *Defa20* (1.6-fold, p=0.0386) gene transcripts were observed in *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice when compared to control animals (Fig. 10A). Although not significant, both sexes showed a tendency of reduction in *Lyz* expression, a hallmark of *Salmonella* Typhimurium infections (Fig. 10).

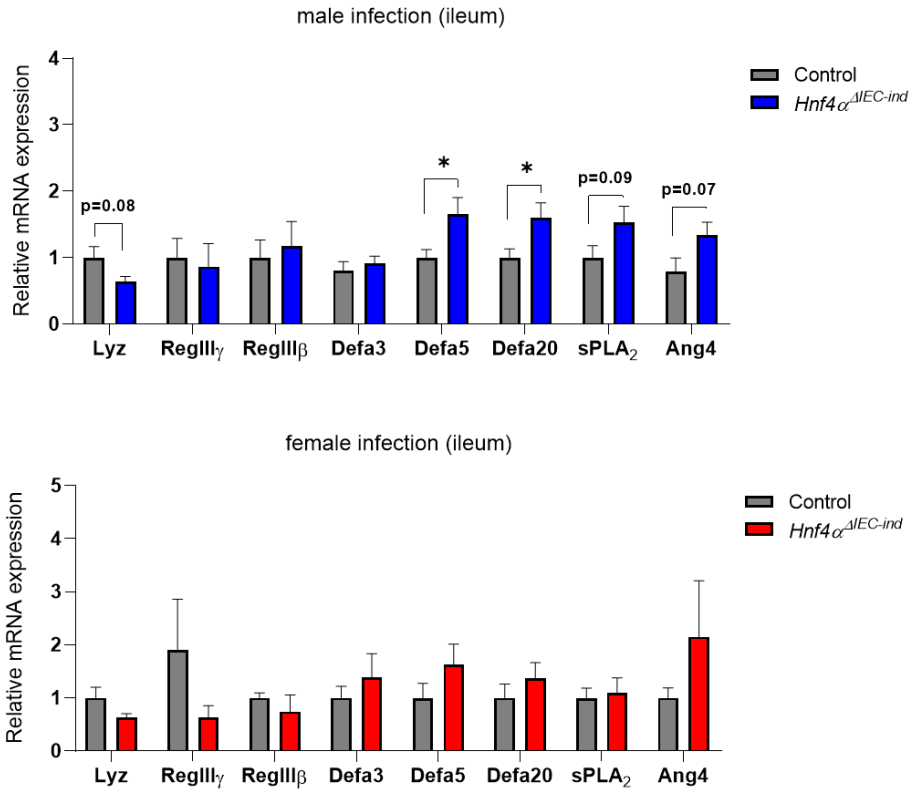


Fig. 10: Profile of AMP genes expression in the ileum of *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice during bacterial infection. qPCR was performed for the quantification of different AMP genes in the ileum of control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ male (A) and female (B) mice. Expression levels are shown as mean values (± SEM) relative to controls (n=6-8 mice per group) and were normalized with *TBP* housekeeping gene transcripts. For each graph, an *unpaired t-test* or *Mann-Whitney* test was used to measure significance.

3.4.4 Intestinal sIgA production

The sIgA is identified as the most abundant intestinal immunoglobulin (Ig), with an estimate of approximately 3 g/day of this molecule being secreted in the intestine of healthy individuals (Rogier et al., 2014). Intestinal sIgA displays a polymeric structure, which not only distinguishes it from other Ig variants, but also contributes to efficient transport through the epithelium, secretion, and finally recognition and elimination of pathogens (Pabst & Slack, 2020).

We performed ELISAs from ileum and colon homogenates of infected animals to verify if bacterial infection of *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice was correlated with changes in intestinal IgA levels. The results obtained were extrapolated against a standard curve prepared with known murine standard IgA concentrations. As observed in Fig. 11, no significant difference was detected in the sIgA concentrations among the different infected groups of mice.

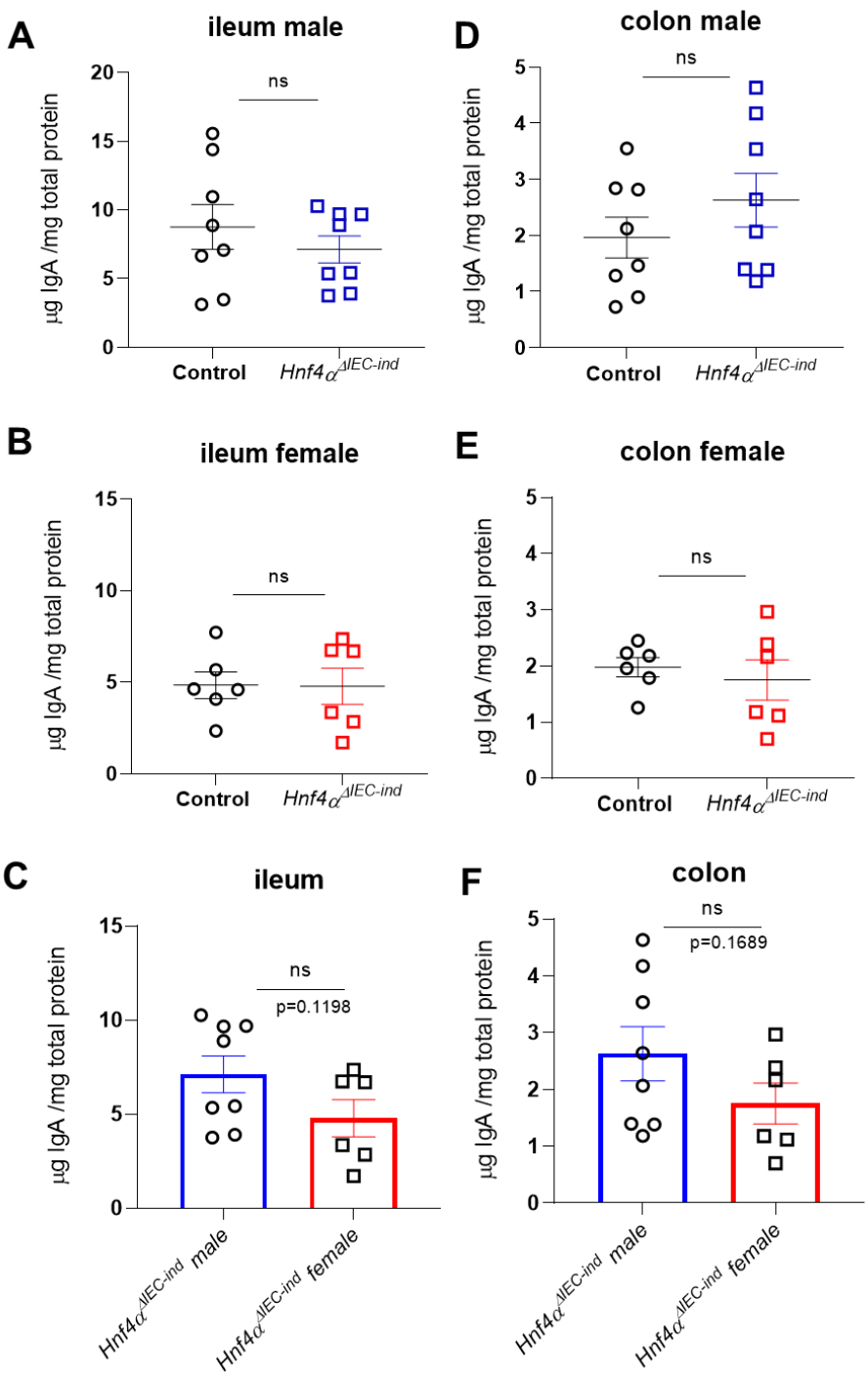


Fig. 11: Quantification of total sIgA production in the intestine of mice after enteric infection. sIgA concentration in luminal/epithelium contents from the ileum of male (A) or female (B) and from the colon of male (D) or female (E) of infected *Hnf4a*$^{\Delta IEC\text{-}ind}$ and control mice was determined by ELISA. A comparison between both sexes of infected *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice was done for the ileum (C) and the colon (F). Expression levels are shown as mean values (± SEM), (n=6-8 mice per group). For each graph, an *unpaired t-test* was used to measure significance.

3.4.5 Mucus layer

Among the essential functions of Goblet cells are: 1) sensing of luminal antigens through the activation of various receptors or molecules (TLR, Myd88 or the Nlrp6 inflammasome), which lead to endocytosis/elimination of these luminal threats; 2) synthesis and secretion of glycoproteins (mucin) and other factors to constitute a semipermeable mucus layer functioning as a dynamic protective barrier in capturing and limiting the passage of microorganisms (Zhang and Wu, 2020). A damaged mucus layer can lead to a more efficient infection of the intestinal mucosa by pathogens.

Therefore, we decided to evaluate the biochemical-structural quality of mucus by analyzing the expression of different genes that participate in its biosynthesis (*Muc1-3*, *Retnlb*, *Tff3*, *Fcgbp*, and *Agr2*). A qPCR quantification analysis revealed a significant increase in the expression of *Retnlb* gene transcripts, that encode for a bactericidal protein, in the ileum (10-fold, p=0.0379; Fig. 12A) and colon (2.4-fold, p=0.0063; Fig. 12C) of *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice. This effect was also observed in the colon of *Hnf4α*$^{\Delta IEC\text{-}ind}$ female mice (2.7-fold, p=0.0469; Fig 12B).

We also observed an upregulation of *Muc2* gene transcripts during early infection events in the ileum of *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice (2.1-fold, p=0.0009; Fig. 12C) when compared with their respective controls. On the other hand, a significant reduction of *Muc3* gene transcripts expression (1.5-fold, p=0.0239) was observed in the colon of *Hnf4a*$^{\Delta IEC\text{-}ind}$ female mice when compared to controls (Fig. 12B).

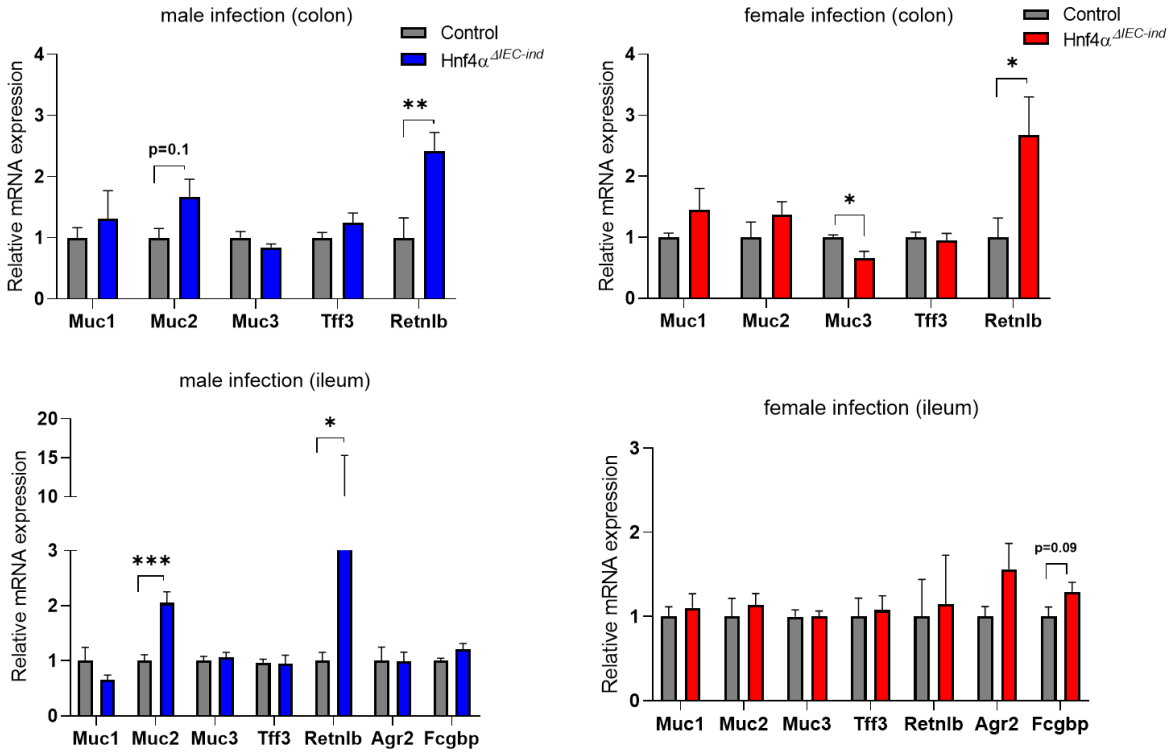


Fig. 12: Altered gene expression for intestinal epithelial goblet cells of *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice after infection with invasive deficient *Salmonella* Typhimurium. Goblet cell gene transcripts were quantified by qPCR in the colon of male (A) and female (B) control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice as well as in the ileum of male (C) and female (D) control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice. Expression levels are shown as mean values (± SEM) relative to controls (n=6-8 mice per group) and were normalized with the *TBP* housekeeping gene transcripts. For each graph, an *unpaired t-test* or non-parametric *Mann-Whitney* test was used to measure significance.

3.5 Hnf4a$^{\Delta IEC\text{-}ind}$ mice display increases in the small intestinal number and size of goblet cells after attenuated *Salmonella* infection.

Based on our qPCR analysis and on the ability of *Salmonella* Typhimurium to affect goblet cells number in the intestinal epithelium (Arnold et al., 1993) (DH Kulkarni et al., 2018), we examined whether *Hnf4a* gene deletion affected intestinal epithelial histology after infection. No major histological change was observed from H&E staining of ileal (Fig. 13A and B) and colonic (Fig. 14A and B) sections obtained for all mouse groups. Alcian Blue/PAS staining revealed a global increase in the number of goblet cells in the ileum of infected *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice (Fig. 13C and D), an observation that was not recapitulated in the colon of these mice (Fig. 14C and D). Goblet cells counting confirmed a 2.5-fold increase in the number of cells per ileal crypts (p=0.0002; Fig. 15A) and a 1.3-fold increase per ileal villi of *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice (p=0.0295; Fig 15B). In addition, a 1.4-fold increase (p=0.0001; Fig. 15C) in the

average of goblet cell size was detected along the entire length of the ileal crypt-villus axis of infected *Hnf4α*$^{\Delta IEC\text{-}ind}$ male mice when compared to infected control mice. Intriguingly, *Hnf4α*$^{\Delta IEC\text{-}ind}$ female mice showed a 1.7-fold increase in the number of goblet cells per ileal crypts (p=0.0060; Fig. 15B) while no change was observed in the number of cells per villi (Fig. 15A). Additionally, no significant change was observed in goblet cell size in the ileum of these mice (Fig. 15D). In contrast to the ileum observations, no apparent change was visualized for goblet cells phenotype in the colon of both male and female *Hnf4α*$^{\Delta IEC\text{-}ind}$ infected mice when compared to controls (Fig. 14C, D).

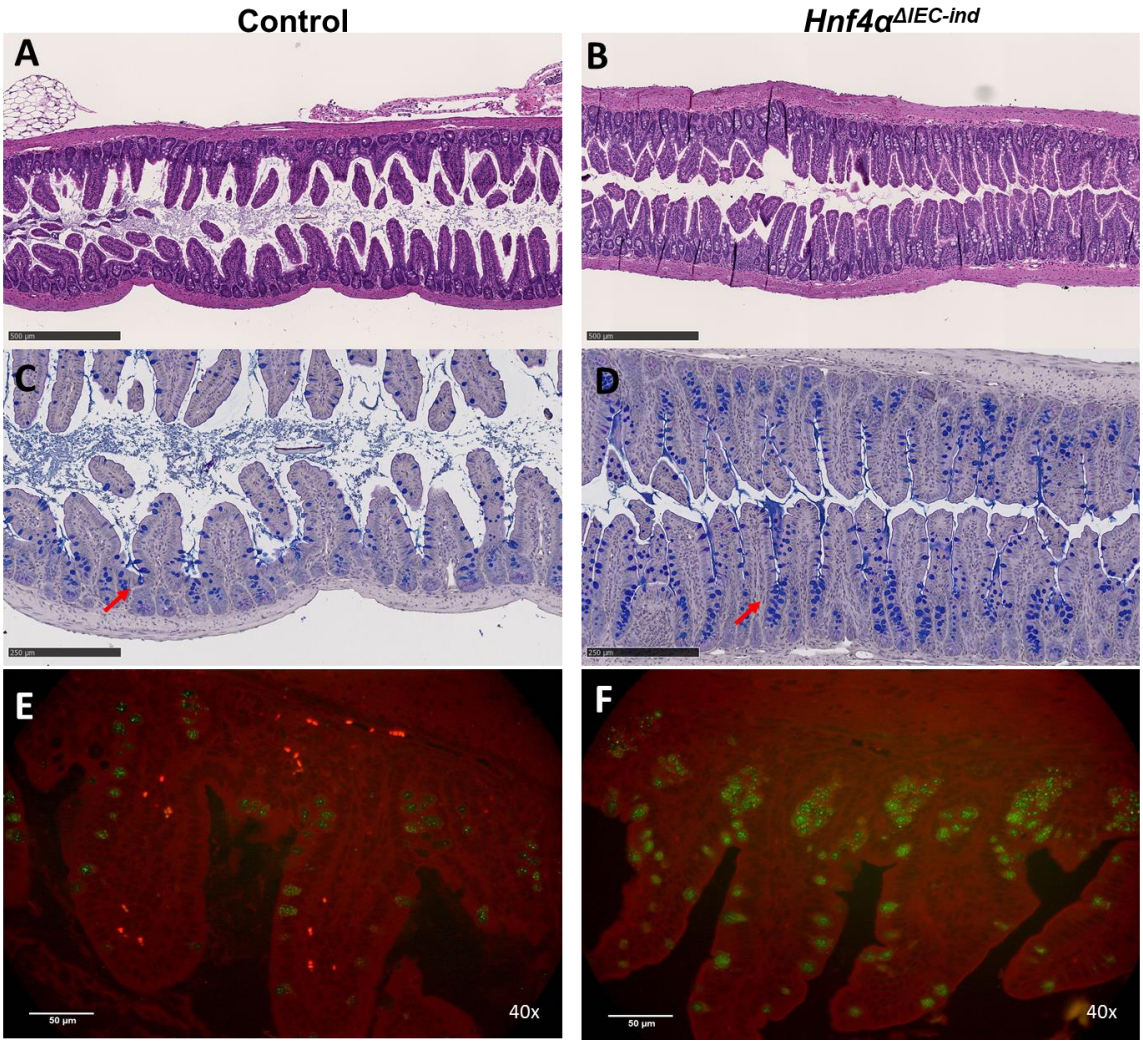


Fig. 13: Histological and goblet cells analyses in the ileum of *Hnf4α*$^{\Delta IEC\text{-}ind}$ mice after *Salmonella typhimurium* strain SB103 infection. H&E staining of ileal sections from control (A) and *Hnf4α*$^{\Delta IEC\text{-}ind}$ (B) infected mice (representative of 6-8 mice per group). Alcian Blue/PAS staining was performed on ileal sections from control (C) and *Hnf4α*$^{\Delta IEC\text{-}ind}$ (D) infected mice (representative of 6-8 mice per group). Scale bar = 250µm. Immunofluorescence of MUC2 was performed on ileal sections from control (E) and *Hnf4α*$^{\Delta IEC\text{-}ind}$ (F) infected mice (n=3 mice per group). Magnification 40x.

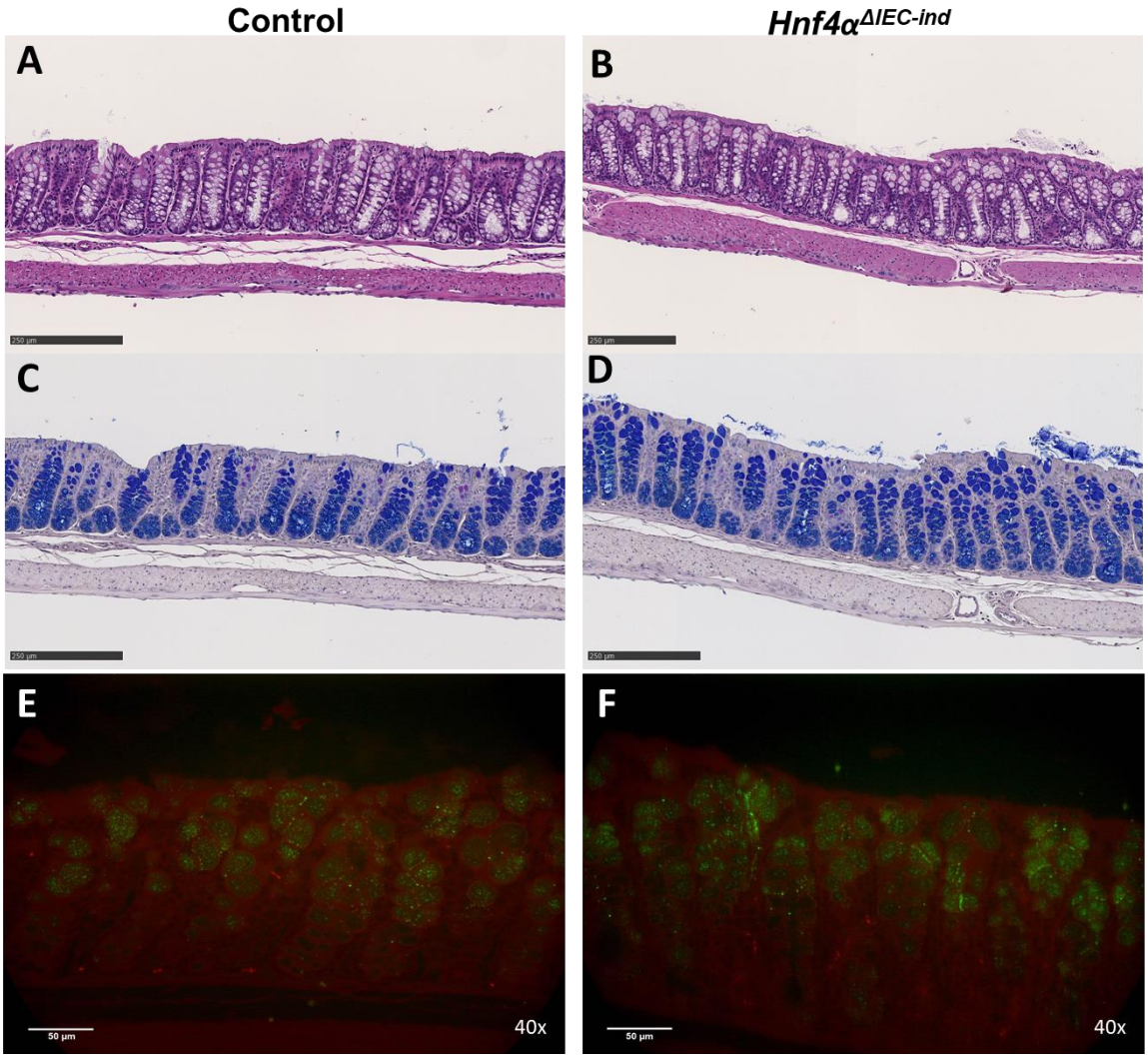


Fig. 14: Histological and goblet cells analyses in the colon of *Hnf4α*$^{\Delta IEC\text{-}ind}$ mice after *Salmonella typhimurium* strain SB103 infection. H&E staining of colon sections from control (A) and *Hnf4α*$^{\Delta IEC\text{-}ind}$ (B) infected mice (representative of 6-8 mice per group). Alcian Blue/PAS staining was performed on colon sections from control (C) and *Hnf4α*$^{\Delta IEC\text{-}ind}$ (D) infected mice (representative of 6-8 mice per group). Scale bar = 250µm. Immunofluorescence of MUC2 was performed on colon sections from control (E) and *Hnf4α*$^{\Delta IEC\text{-}ind}$ (F) infected mice (n=3 mice per group). Magnification 40x.

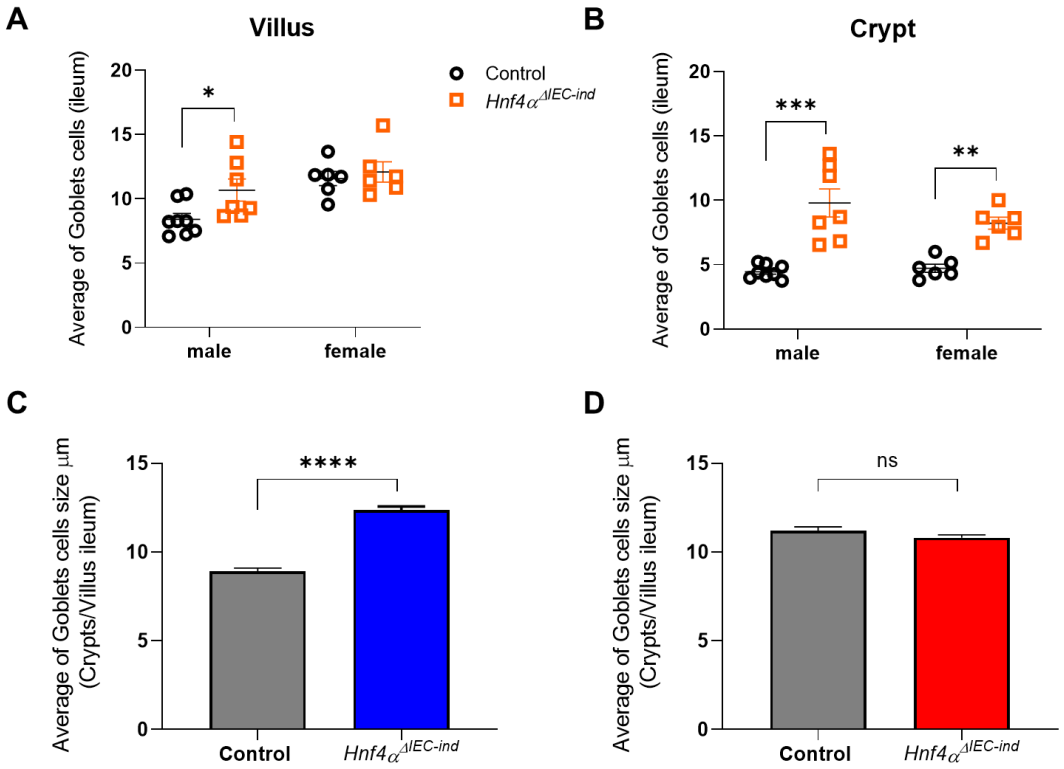


Fig. 15: Altered number and size of goblets cells in the ileum of infected *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice. Average number of Alcian Blue/PAS-positive cells in the ileum of *Hnf4a*$^{\Delta IEC\text{-}ind}$ and control mice per villus (A) or per crypt (B) (20 crypt-to-villus axes calculated from 6 to 8 mice per genotype). Average size of goblet cells in the ileum of male (C) or female (D) *Hnf4α*$^{\Delta IEC\text{-}ind}$ and control mice (5 crypt-to-villus axes calculated for 3 mice per genotype). Statistical significance was calculated using A, B) *one-way Anova* test and C, D) *unpaired t-test* or *Mann-Whitney* non-parametric test. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001.

To further validate our observations, we performed an indirect immunofluorescence (IF) against the MUC2 glycoprotein, the main component produced by goblet cells into the mucus layer. In general, we found a weak labeling of this mucin in the ileum of control infected male mice (Fig. 13E) as opposed to a strong detection of MUC2 in goblet cells from *Hnf4a*$^{\Delta IEC\text{-}ind}$ infected male mice (Fig. 13F). IF analysis in colonic tissues appeared to show a more pronounced expression of MUC2 in *Hnf4a*$^{\Delta IEC\text{-}ind}$ infected male mice when compared to control mice (Fig. 14E and F).

3.6 Deletion of HNF4α in the ileum of male mice impact gene transcript levels associated with secretory cells specification and differentiation.

Histological analysis of *Hnf4a*$^{\Delta IEC\text{-}ind}$ infected mice showed important differences in the number and size of ileal goblet cells in the crypts of Lieberkühn. It is precisely at the bottom

of the intestinal crypt that stem cells ensure an equilibrium in cell turnover and differentiation into absorptive or secretory cells (Van Der Heijden and Vermeulen, 2019).

To determine if the loss of HNF4α could influence epithelial cell fate, total RNA from the ileum of control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice was isolated and further analyzed by qPCR (Fig. 16). We found significant changes in the expression of genes associated with goblet cells differentiation and maturation, with higher expression for *Math1* (1.4-fold, p=0.0077) and *Spdef* (1.3-fold, p=0.0250) in *Hnf4a*$^{\Delta IEC\text{-}ind}$ infected male mice when compared with the control group (Fig. 16A). These observations were not recapitulated in the ileum of *Hnf4a*$^{\Delta IEC\text{-}ind}$ infected female mice (Fig. 16B).

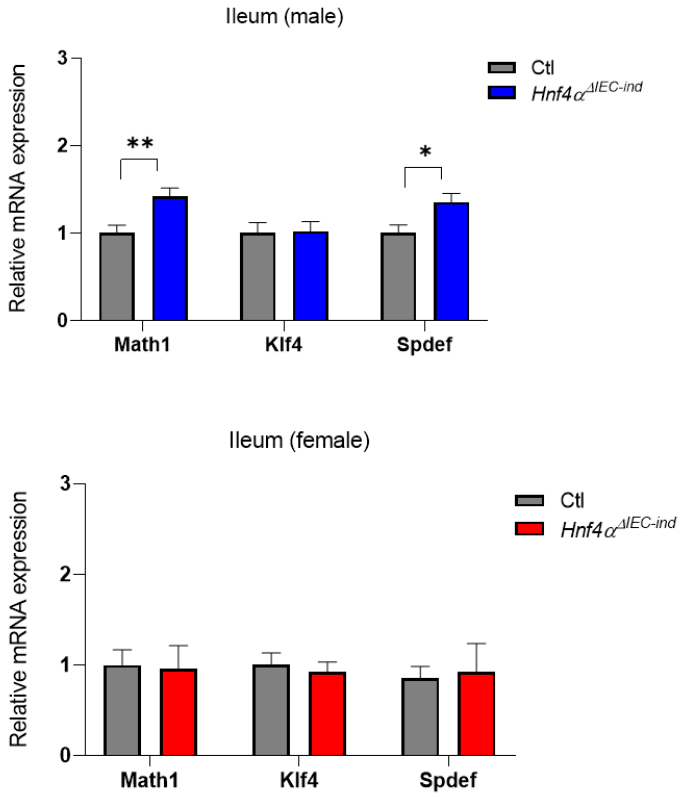


Fig. 16: Loss of HNF4α enhance the expression of genes associated with goblet cells differentiation in the ileum of *Hnf4a*$^{\Delta IEC\text{-}ind}$ infected male mice. Relative gene transcript levels for goblet cell differentiation were analyzed by qPCR in the ileum of male (A) or female (B) infected *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice. Fold change represents the normalization of expression values compared to *TBP* housekeeping gene transcripts (n=6-8 mice per group). Statistical significance was calculated using an unpaired *t-test*. *P<0.05, **P<0.01

3.7 Loss of HNF4α seems to influence fucosylation patterns of infected female mice in the small intestine.

It is known that changes in glycosylation patterns of a host organism can modulate the susceptibility to enteropathogenic infections (Mottram et al., 2017). More specifically, α-1,2-fucosyltransferase is responsible for the incorporation of terminal fucose residues in the mucus glycoproteins, thus contributing to their maturation and correct functioning. This glycosyltransferase is encoded by the *FUT2* gene, which was identified as a CD risk locus (Tong et al., 2014). Bearing this in mind, we conducted a study of post-translational modifications, more specifically on fucosylation, in the intestinal epithelium of our experimental mice model after infection with *Salmonella* Typhimurium SB103. Analyses of the ileum of both control and *Hnf4a*$^{\Delta IEC\text{-}ind}$ animals were performed by quantifying the relative expression of *Fut2* mRNA by qPCR, as well as by IF against the *lectin Ulex europaeus agglutinin-1* (UEA-1), that recognizes α-1,2-fucose present in glycoconjugates (Fig. 17).

As observed in Fig. 17A, none of the evaluated Hnf4a$^{\Delta IEC\text{-}ind}$ groups showed significant differences in the relative levels of *Fut2* transcripts when compared to the control groups. However, a tendency to increased expression was observed in the ileum of *Hnf4α*$^{\Delta IEC\text{-}ind}$ female mice when compared with their controls. Similarly, immunolocalization analysis showed that the infected control female group displayed a restricted pattern of fucosylation in the bottom of the ileal crypts, while colonized *Hnf4a*$^{\Delta IEC\text{-}ind}$ female mice displayed a much wider pattern of fucosylated epitopes in Paneth and goblet cells (Fig. 17B).

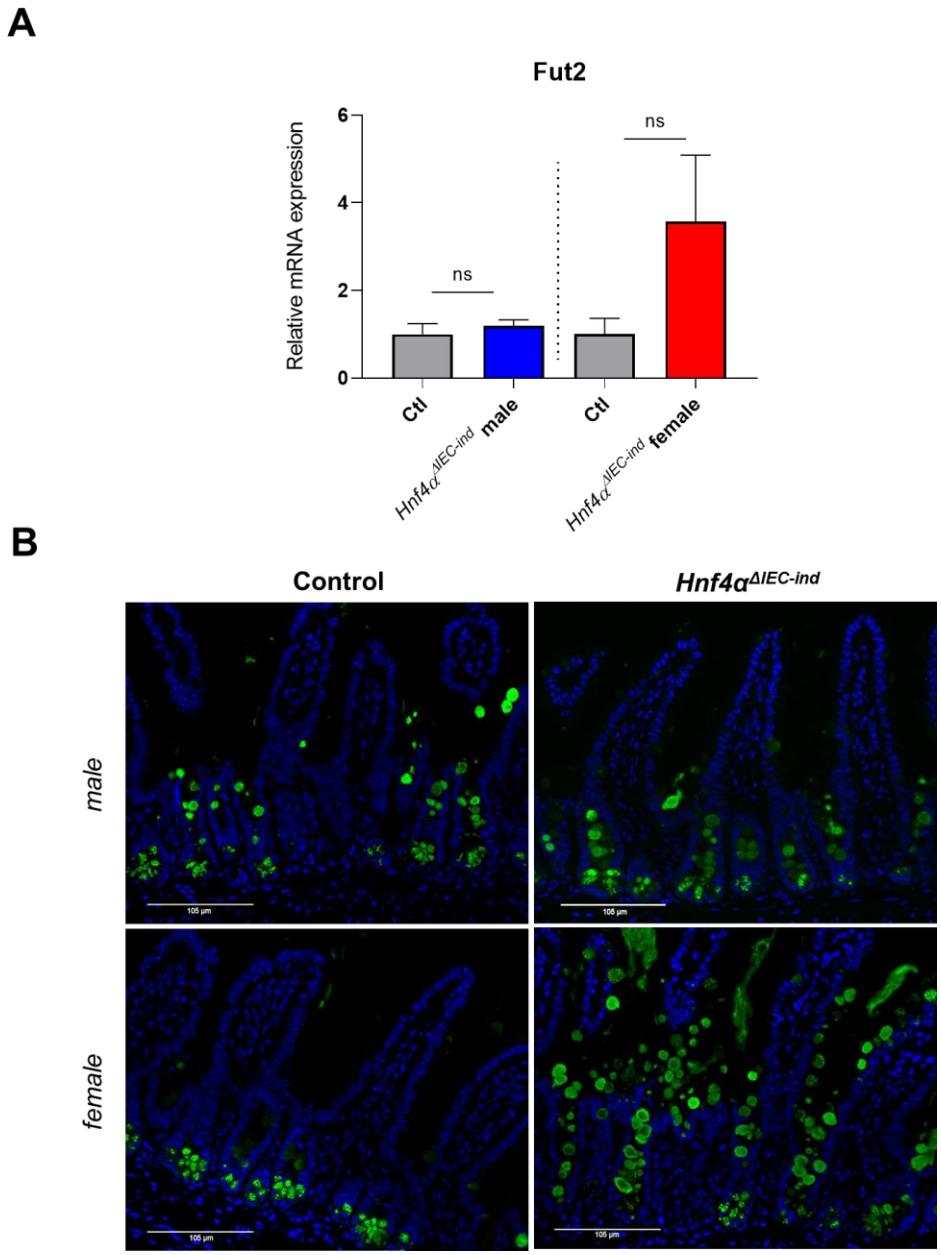


Fig. 17: Loss of HNF4α promotes abnormal fucosylation in infected mice. A) Relative expression of *Fut2* gene transcripts was quantified by qPCR. Fold change represents the normalization of expression values compare to *TBP* gene transcripts used as housekeeping gene. B) Fucosylated residues was studied by UEA-I (green) lectin immunofluorescence. Infected *Hnf4a*$^{\Delta IEC\text{-}ind}$ female mice showed an increase of intestinal fucosylation when compared to controls. Nuclei were counterstained with DAPI (blue). Magnification 20x (n=3 mice per group).

4. DISCUSSION

One of the fundamental properties of the intestinal mucosa is its barrier function, acting as a wall between the immune system and antigens that come from nutrients or the commensal microbiota (Maynard et al., 2012; Allaire et al., 2018). The barrier is formed by different elements that contribute to the maintenance of the host's homeostasis, such as the simple layer of specialized cells (enterocytes, Paneth cells, goblets cells and endocrine cells), the mucus layer that covers and protects the epithelium, and immunity cells found in the lamina propria (Halpern and Denning, 2015). These components are strongly regulated in order to control the transport of ions, water, and molecules from the lumen to the underlying tissues (Groschwitz and Hogan, 2009; Shen et al., 2011). However, loss of cellular surface integrity can cause excessive translocation of materials and pathogens leading to the activation of different pathways or mechanisms of inflammation in the mucosa. A defective barrier has been described as one of the possible causes or consequences in the appearance of chronic inflammatory disorders in humans, including UC and CD, which are classified as IBD (Antoni et al., 2014; McGuckin et al., 2009; Michielan and D'Incà, 2015; Chang et al., 2017). On the other hand, a number of molecules associated with IBD susceptibility have been identified in close relationship to the dynamic interaction complex established by the commensal microbiota - the immune system and the host environment (Liu et al., 2015; Jostins et al., 2012; Graham and Xavier, 2020). Among them, stand out NRs such as the Glucocorticoid receptor (GR), Peroxisome Proliferator-Activated receptors (PPARγ) or Farnesoid X receptors (FXR), as a group of transcriptional regulators that have become attractive therapeutic targets for the treatment of the disease (Klepsch et al., 2019). In addition to these NRs are the Estrogen receptor (ER), Vitamin D receptors (VDR), Pregnane X receptor (PXR) and Nuclear orphan receptor (NR2F6) that all influence epithelial barrier properties by mediating different processes such as mucus secretion, TJ proteins expression, autophagy and production of goblet and Paneth cells (Wada-Hiraike et al., 2006; Fujita et al., 2008; Garg et al., 2016; Su et al., 2016; Du et al., 2015; Klepsch et al., 2018). Previous GWAS-based work identified *HNF4A* as one of the most enriched risk genes in IBD patient samples (Barrett et al., 2009). *HNF4A* codes for the nuclear receptor HNF4α, an important regulator of cell proliferation and differentiation, as well as other metabolic events in both the liver and intestine (Babeu et al., 2009; Babeu and Boudreau, 2014). The strong decrease

in expression of this gene is shown as a distinctive feature in IBD patients, thus suggesting a possible protective role for this TF in this context (Ahn et al., 2008; Darsigny et al., 2009). However, despite numerous studies in the liver, there is little clarity about the effects of HNF4α on the intestinal barrier and thus their relationship to inflammation (Battle et al., 2006; Parviz et al., 2003; Prager et al., 2015; Lussier et al., 2008). Some authors have suggested that in mice devoid of intestinal HNF4α, changes in the epithelium were associated with an increased intestinal permeability (Cattin et al., 2009). However, previous work in our laboratory only evidenced restrictive changes at the ions transport level (Darsigny et al., 2009).

To further understand how the loss of HNF4α can influence barrier function, we eliminated the expression of this gene at the intestinal epithelial level using a recombination system inducible by Tamoxifen (*Villin*-CreERT2). Our *Hnf4α*$^{\Delta IEC\text{-}ind}$ mouse model showed that the *Villin*-CreER system presented a high recombination efficiency as determined by measuring *Hnf4α* relative transcripts remaining levels. CreER transgenic technology is widely used in genetic knockout approaches and is based on the expression of the Cre recombinase enzyme fused to a point mutated G525R estrogen receptor. Under physiological conditions, the estrogen receptor is not able to bind to its natural ligand 17β-estradiol, so the enzyme is only activated and translocated to the nucleus in the presence of an inducing molecule, TAM or its intermediate metabolites (Donocoff et al., 2020). Unlike the conditional Cre/LoxP recombination system, the inducible model not only allows to carry out the functional study of a gene in a specific tissue, but also allows this to take place at a specific time point (Zhong et al., 2015). In this way, the possible combined long-term effect of embryonic *Hnf4α* deletion with modulation exerted by other factors such as the microbiota or the immune system on overall intestinal barrier properties could be minimized (Martini et al., 2017). A recent study by Rutlin *et al.* that used Rosa26m$^{TmG/+}$ reporter mice with the inducible *Villin*-CreER model revealed the appearance of a diffuse expression of the transgene activated by Cre recombinase enzymatic activity in the small intestine, as well as an isolated expression pattern in some regions of the colon after 7 days of TAM injection (once daily for 5 consecutive days) (Rutlin et al., 2020). However, a previous report by Cattin *et al.* that used the same transgenic model showed between 87% to 90% of *Hnf4α* mRNA reduction as well as a 90% decrease of protein in the jejunum of mutant mice, 30 days after TAM injections.

Similar values were observed in other intestinal segments of HNF4α null mice when compared with control animals (Cattin et al., 2009). Similarly, our data supports an effective deletion of *Hnf4a* after only 7 days of Cre recombinase induction in both the ileum and colon. A possible explanation between the differences observed in our results and the weak expression detected for the enzyme CreER in intestinal tissue (Rutlin et al., 2020) may be related to the ability of HNF4α to negatively autoregulate its own promoters (Schwartz et al., 2009). Consequently, the initiation of the gene deletion process in the IEC nucleus can constitute a signal strong enough to provoke a cycle of self-elimination and therefore generate a significant decrease in the relative expression of mRNA.

In our study, the impact of *Hnf4a* deletion in the intestinal epithelium was initially evaluated by a paracellular permeability assay, using the fluorescent macromolecule FITC-dextran (4 kDa). Paracellular permeability allows to measure the functionality of the epithelial barrier through the flow of molecules that cross the intestinal wall. These markers of different sizes are selected based on their capacity of not being metabolized in the intestine and on their capacity to be rapidly transported to the bloodstream or urine after passage through a permissive epithelium (Bischoff et al., 2014; Vancamelbeke and Vermeire, 2017). Contrary to controls, $Hnf4a^{\Delta IEC\text{-}ind}$ mice of both sexes showed an increase in the concentration of FITC-dextran in the serum, thus indicating that the absence of HNF4α seems to be associated with an increase of permeability of the mucosal barrier. Other studies have also highlighted changes in intestinal permeability both *in vitro* and *in vivo* in the absence of HNF4α (Ahn et al., 2008; Cattin et al., 2009). However, this technique did not allow us to accurately specify the region or segments of the intestine that may be affected.

The integrity of the epithelial barrier is determined by the cell-cell contacts that are established in the tissue. These intercellular junctions are made up of AJ, desmosomes, and mainly TJ, which not only guarantee the mechanical cohesion of the cells and their correct polarization but also allow the semipermeable sealing of the apical and basal lateral region of the cells (Allam-Ndoul et al., 2020). The TJ are fundamentally supported by the differential presence and physiological characteristics of the Claudin proteins family. Although the vast majority of claudins are responsible for re-establishing the barrier, others directly regulate the passage of water and ions through the mucosa (Günzel and Yu, 2013; Amasheh et al., 2002; Rosenthal et al., 2017). For the proper functioning of the intestinal

epithelium, a balance is necessary between proliferation, differentiation and apoptosis. Loss of HNF4α is associated with the expression of oxidative stress genes, appearance of apoptosis, and alteration of the intestinal epithelial architecture (Darsigny et al., 2009; Darsigny et al., 2010). All these elements, together with changes in the TJ proteins distribution and increased intestinal permeability, can contribute to an invasion of the epithelium mediated by the commensal microbiota itself or by pathogenic microorganisms. To explore this hypothesis and evaluate the passage of bacteria into the underlying tissues via the paracellular pathway, we performed infection experiment using an invasion-deficient strain of *Salmonella* Typhimurium (SB103). We decided to focus on colon and ileum sections, since they constitute the sites in the GI tract where the greatest number of bacteria is concentrated while the protective functions of the epithelial barrier must be reinforced in these segments. In early infection events, *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice exhibited bacterial loads in the intestinal mucosa and in the organs with a systemic invasion similar to the control animals. Besides, all groups of infected mice showed a considerable decrease in the *Salmonella* Typhimurium burden on day 2 pi as measured in stools. Under normal conditions, *Salmonella* needs to adhere to the mucus layer in order not to be expelled from the intestine (Furter et al., 2019). The observed effect on day 2 pi may be related to the fact that we are using a non-invasive bacterium, which in addition to presenting the M cells and DC sampling of the lumen as an entry mechanism, probably requires an epithelial damage in order to have a faster and more effective invasion. Also, the delay required for this process to take place depends on how quickly the bacteria pass through the mucus layer (Furter et al., 2019), as well as the extent of the mucosal damage. Rapid mucus turnover in the distal colon (1-2 h) is known to help eliminate microbes found in the intestine (Johansson, 2012; McLoughlin et al., 2016). The ileum presents a thinner layer of the mucus hydrogel than the colon and thus tends to detach easily (Atuma et al., 2001; Ermund et al., 2013). Therefore, we can assume that during the process of eliminating the feces, the bacteria that were trapped in the mucus were washed away thus contributing to the decrease in the number of bacteria.

In agreement with the changes in intestinal permeability, the infected *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice showed distension of the intercellular space between IECs. Male *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice also showed significant overexpression of *Cldn2* gene transcripts in the colon, while females exhibited downregulation of *Cldn7* and increase of *Cldn4* gene transcripts. CLDN2 is a

protein that forms pores in the plasma membrane and its abundant expression is associated with the loss of intestinal epithelial functions, increased permeability and a high correlation with inflammatory diseases such as UC and CD (Weber et al., 2008; Das et al., 2012; Zeissig et al., 2007). On the other hand, CLDN7 deficiency in the colon triggers an increase in the paracellular flow of solutes and induction of inflammation in mice (Tanaka et al., 2015). Farkas *et al*. also demonstrated that HNF4α acts as a positive regulator of CLDN7 expression during IEC differentiation (Farkas et al., 2015). Our data correlate with previous observations in the literature (Cattin et al., 2009) and suggest that indeed, HNF4α is capable of modulating the expression of tight junction components related to permeability. However, in the present study, we cannot fully conclude the impact of such gene modulation without first carrying out an analysis of the protein profile for these genes.

Additionally, we observed that ileal alterations differed from those observed in the colonic tissue. *Hnf4a*$^{\Delta IEC\text{-}ind}$ males showed downregulation of *Cldn3* expression in the ileum, as well as a certain trend toward *Cldn15* downregulation, while only a reduction of this latter was detected in *Hnf4a*$^{\Delta IEC\text{-}ind}$ female mice. Decrease in *CLDN3* was observed in colonic biopsy samples from IBD patients (Prasad et al., 2005). This claudin, as well as claudin-1, -4, -5, -8, is associated with the formation of strands in the membrane of IECs and tighten TJ, so changes in its expression pattern can lead to increase in intestinal permeability (Bücker et al., 2010). On the other hand, CLDN15 is predominantly expressed in the small intestine and its stimulation of expression allows the sealing of the paracellular space and selective channels formation for the transport of cations in the membrane (Samanta et al., 2018). In line with our observations, reduction in CLDN15 expression and impairment of ion transport in absence of HNF4α was detected in the colon of mice with features associated with inflammation (Darsigny et al., 2009). In this sense, Muthusamy *et al.* also showed a direct correlation both *in vitro* and *in vivo* with the deletion of *HNF4A* and reduced expression of the transporter Na^+/ H^+ exchanger isoform 3 (NHE3), both at the gene transcript and protein levels. Furthermore, site-specific mutagenesis assays within the HNF4α interaction motif in the *NHE3* promoter region showed a direct relationship between this TF and transcriptional regulation of this gene (Muthusamy et al., 2018). NHE3 is central in mediating coupled electroneutral NaCl absorption and its reduced expression was associated with IBD and enteric infections (Priyamvada et al., 2015; Gurney et al., 2017). In addition, expression of

CLDN15 is essential for the Na^+/glucose cotransport (SGLT) through the enterocyte extracellular membrane (Tamura et al., 2011). The activation of this cotransporter constitutes a sufficient stimulus to induce an increase in the density of pores in the intestinal epithelium and therefore in the permeability to small solutes. SLGT controls and regulates the plasticity of TJ through the activity of the myosin light chain kinase (MLCK) (Turner et al., 1997; Turner et al., 2000; Clayburgh et al., 2004). Several studies propose a close link between SLGT, MLCK, and the NHE3 transporter, suggesting that the activation of the latter occurs downstream of SLGT, although this mechanism has not been fully elucidated (Hu et al., 2013). Based on the literature, we could speculate that in addition to create a breach in the epithelial barrier, the decrease in *Cldn15* constitutes a defense mechanism in the regulation of permeability in the absence of HNF4α in the intestine. Similarly, it would be interesting to investigate whether HNF4α is capable of modulating the expression of other genes such as *MLCK*, since loss of the barrier mediated by this kinase can have effects similar to the activation of the immune system in diseases such as IBD (Zuo et al., 2020). Indeed, *MLCK* knockout mice were shown to be more protected during experimental colitis (Su et al., 2013). Therefore, our results indicate that the increase in paracellular permeability or the modulation of ionic transport as previously reported (Darsigny et al., 2009) are not mutually exclusive in terms of HNF4α regulatory functions on the intestinal epithelial barrier. To this end, compensatory external factors probably intervene for the overall regulation of these processes in the *in vivo* conditions. The interactions between the microbiota and the barrier play an essential role in the proper functioning of the intestine (Parekh et al., 2015). Bacteria and their metabolites, such as SCFA, act by positively or negatively modulating the activation of different NRs, such as PXR, FXR, or PPARγ, which protect against mucosal inflammation or pathogens invasion (Ranhotra et al., 2016; Byndloss et al., 2017). A meta-analysis carried out in zebrafish by Davison *et al.* and also corroborated in murine IECs, showed that the commensal microbiota is capable of suppressing the expression of HNF4α in the epithelium, affecting the expression of dozens of genes that are usually activated by this NR with the result of promoting patterns of gene expression associated with IBD (Davison et al., 2017).
Contradictory to what was expected, variations in the expression of some TJ components in *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice were not enough to generate an acute increase in *Salmonella* infection. Several factors may be influencing this result, including the possible compensatory action of

HNF4γ in the intestinal epithelium. Indeed, one report has suggested that there is a genetic and functional redundancy between *Hnf4a* and *Hnf4g* paralog genes in the intestine (Chen et al., 2019a). Another element to take into account is the molecular size of the pores that form in the IECs membrane. In a study carried out by Van Itallie *et al.* they analyzed the polyethylene glycol molecules flow crossing the ileum of pigs or cells monolayers from the intestine and kidney. They showed that the increase in the expression of CLDN2 in the epithelium implies an increase in the number of pores, but not in their size. Molecules larger than pores were not detected despite a permeability alteration (Van Itallie et al., 2008). The small intestine is considered to be the site of entry and colonization of many enteropathogenic bacteria in humans and animals that also include *Salmonella* Typhimurium (Garner et al., 2009). The epithelial structure of this segment is governed by the crypt-villus axis, for which a permeability gradient was determined based on the diameter of the observed pores. Indeed, villi are composed of small pores (12 Å) whereas crypts display large pores (100 to 120 Å) (Fihn et al., 2000). Two pathways allow the passage of nutrients and water through the TJ. These routes differ in terms of load, size and capacity. The pore pathway has a high conductance and is very size-selective, allowing the transport of substances between 6 to 8 Å in diameter. The leak pathway is less specific since macromolecules with a size of up to approximately 100 Å are able to cross it (Buschmann et al., 2013). Considering this, the polysaccharide dextran (4 kDa) used to measure paracellular flux in our analysis is much smaller than bacteria since only one of the outer membrane proteins of *Salmonella* Typhymurium contains a molecular mass of 49 kDa, which is 12 times higher than that of the fluorescent marker (Hamid and Jain, 2008). We then assume that the passage of only small solutes through the barrier is probably favored in *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice. Therefore, it is possible that the absence of HNF4α mainly mediates intestinal permeability directed by the tight junction pore pathway. CLDN2 plays a fundamental and complex role in intestinal homeostasis, which has not been fully elucidated. Although colonic-forced expression of CLDN2 led to an intensification in intestinal permeability in transgenic mice, this effect was also correlated with amplified levels of colonocyte proliferation and protection against colitis-induced cell death coupled to a possible association with immunogenic tolerance (Ahmad et al., 2014). On the other hand, it is known that CLDN4 expression decreases the paracellular permeability to Na^+ (Van Itallie et al., 2001). Increase of *Cldn4* transcripts and

protein levels were observed in the colon of *Hnf4a*-null mice (Darsigny et al., 2009), as well as in IBD patients (Weber et al., 2008). Our *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice showed similar results regarding the relative expression of mRNA for this gene, and this is an indication of how HNF4α contributes to preserving and improving the function of the intestinal epithelial barrier.

Despite the low systemic bacterial load observed in our infection model, inflammation was an important aspect to analyze since it is involved in protecting the body against the presence of invading microbes (Broz et al., 2012). Out of a total of 9 genes encoding cytokines and other inflammatory mediators, female *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice only showed a decrease in *Casp1* mRNA expression in the ileum and colon. Invasion and proliferation of *Salmonella* Typhimurium cause activation of the intrinsic inflammasome in IECs, a proteolytic complex related to cell death induced by inflammation and commonly referred to pyroptosis. The inflammasome requires caspase-1 for cleavage and maturation of pro-cytokines such as IL-18 or IL-1β. However, to allow secretion of these cytokines, formation of pores in the cell plasma membrane through gasdermin D processing (GsdmD) is required for the expulsion into the lumen or lysis of the infected enterocytes to lead to bacteria elimination (Wang et al., 2019). Crowley *et al.* observed that caspase-1 plays an important role in mediating the antimicrobial response in the early events of bacterial replication. Interestingly, these authors also suggested that a decrease of this molecule expression occurs during the infection course (Crowley et al., 2020). Parallel to this study, we observed a tendency toward an increase of fucosylation in the ileal tissue of *Hnf4a*$^{\Delta IEC\text{-}ind}$ female mice. Goto *et al.* demonstrated that mice deficient in *Fut2* presented with high inflammation and susceptibility to *Salmonella* infection (Goto et al., 2014). It is known that fucosylation of proteins at the IECs surface is a determining phenomenon in the protection against *Salmonella* Typhimurium. Fucose residues incorporated into the terminal galactose on glycans can affect the expression of metabolic pathways and virulence genes in microorganisms (Pham et al., 2014; Pickard et al., 2014). Considering our results, we speculate that *Hnf4a*$^{\Delta IEC\text{-}ind}$ female mice would present a slightly more active invasion of *Salmonella* when compared to the rest of the infected animals. However, in the absence of HNF4α, there are other mechanisms that allow the infection to be partially resolved, at least at similar levels to those detected for control mice. Recently, Suwuandi *et al.* used a *Salmonella* Typhimurium ΔaroA mutant strain that

produces pathological damage restricted at the intestinal mucosa level. They detected that during the first 3 days pi, *Fut2*$^{+/+}$ and *Fut2*$^{-/-}$ mice showed similar bacterial colonization. However, at 7 days pi, *Fut2*$^{+/+}$ mice developed a considerable increase in colonic infection and inflammation. To explain this phenomenon, they argued that fucose and its metabolites constitute an energy source for *Salmonella* and bacterial binding to fucosylated cells occurred through the adhesion protein encoded by the *std* operon (Suwandi et al., 2019). This result is an interesting aspect to take into account in future analyzes.

On the other hand, *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice exhibited a reduction of *IL-18* gene transcripts in the ileum. IL-18 is produced by both immune cells and IECs (Dinarello and Fantuzzi, 2003). In a study of infection in mice with *Salmonella* Typhimurium, Muller *et al.* revealed that unlike IL-1β, the increase of IL-18 in cecum mucosa was only detected at the protein level without any change at the gene transcript levels (Müller et al., 2016). Furthermore, they suggested that IL-18 reduction generates a late stimulation, but not complete attenuation of the inflammatory response to infection (Müller et al., 2016). IL-18 deficient mice are more susceptible to *Salmonella* infection (Raupach et al., 2006). In this sense, our results suggested a less pronounced tendency to systemic infection in *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice when compared to control animals. This observation could mean that: 1) a decrease in *IL-18* could constitute a defense mechanism at the barrier following the loss of intestinal HNF4α. In support of this, mice made deficient in IL-18 production showed a reduction in colitis during the early events of the infection (18 h pi). When compared with control animals, they exhibited a high level of IL-18 protein at 18 h pi, but subsequently decreased to the baseline level at 36 h pi. The authors of this study also found that injection of IL-18 into the mucosa in the presence of an avirulent strain of invasion-deficient *Salmonella* (S.Tmavir; *ΔinvG*; sseD::aphT), was not sufficient to induce inflammation in the epithelium. Therefore, IL-18 is necessary and only modulates the inflammatory response in the presence of an acute infection (Müller et al., 2016); 2) The molecular platform of the inflammasome is activated (NLRP3/ NLRC4) and allows epithelial restriction of the infection independently of IL-18 production; 3) *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice are being less permeable to the bacteria due to the presence of other elements in the intestinal barrier. In this sense, we can also affirm that despite the increase in intestinal permeability, immediate loss of HNF4α does not seem to generate an acute inflammation in the epithelium. HNF4α linkage to this phenomenon seems to be the consequence of

cumulative events over time, as it was previously suggested with the long-term manifestation of IBD-like symptoms in a conditional intestinal HNF4α mutant model in which the deletion has occurred at embryonic day 15.5 (Darsigny et al., 2009).

The expression of different components of the mucus layer (IgA, AMP, MUC, etc.) produced or transported by IECs constitutes an essential defense mechanism as part of the biochemical barrier (Faderl et al., 2015). Intestinal sIgA is produced by B cells found in the epithelium and represents the first line of defense in protecting against invasion by pathogens (Martinoli et al., 2007). In our study, infected *Hnf4a*$^{\Delta IEC\text{-}ind}$ and control mice showed no difference in the amount of ileal and colonic sIgA levels. A study carried out in BALB/c mice with tumors secreting intestinal monoclonal IgA specific to *Salmonella* Typhimurium showed protection during an oral invasion with the bacteria (Michetti et al., 1992). The sIgA is secreted into the mucus and through a process of crosslinking and agglutination in the lumen, which eventually surrounds the pathogen for its subsequent elimination (Moor et al., 2017). However, in the case where there is no acute invasion of the intestinal mucosa, the plasma cells are not capable of activating the mechanisms that induce the overproduction of this Ig. In this sense, Vazquez-Torres *et al.* showed that a strain of *Salmonella* Typhimurium invalidated for SPI-1, and therefore invasive deficient, was unable to stimulate sIgA production in the intestinal mucosa (Vazquez-Torres et al., 1999). Also, it is important to note that the data obtained include the concentration of total sIgA for each intestinal segment analyzed, so perhaps the proportion that the specific IgA represents against the attenuated *Salmonella* is not large enough to imply a significant variation in the overall concentration of this immunoglobulin. Therefore, it would be interesting to perform the analysis of the samples against a bacterial antigen such as LPS.

We also observed that *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice presented an increase in the levels of transcripts that encode some AMP, such as *Defa5* and *Defa20*, as well as a tendency to upregulate *Ang4* or sPLA2. Defensins are the largest family of AMP and are subclassified as αDefensin and βDefensin (Gassler, 2017). Production of αDef5 peptides in transgenic mice infected with *Salmonella* Typhimurium was associated with a decrease in translocation and bacterial viability, thus resulting in a high survival of the mice. Also, a recent study carried out by Castillo *et al*. reported a high expression of *Defa20* in the gut of C57BL/6 mice, with a marked prevalence in the distal region of the small intestine (Castillo et al., 2019). On the

other hand, recently Likewise, Ang4 belongs to a family of RNase that have antibacterial (Gram (-) and Gram (+)) and antiviral activity (Bevins and Salzman, 2011). This molecule not only contributes to the homeostasis of the commensal microbiota, but it has also been observed to have strong activity against *Salmonella*, causing the formation of pore-like structures in bacterial cells (Walker et al., 2013). Therefore, our results indicate that the mucus layer of mutant males could be reinforced in the presence of a pathogen. Since Paneth cells are the main producer of AMP in the intestinal epithelium, it would be interesting to determine if the absence of HNF4α implies changes at the morphological or signaling level in this cell type.

Likewise, the study in our infection model revealed a disturbance of several molecules produced and secreted by goblet cells. *Hnf4α*$^{\Delta IEC\text{-}ind}$ female mice showed high levels of *Retnlb* mRNA expression and a reduction of *Muc3* transcripts in the colon. RELMβ is a bactericidal protein induced by the commensal microbiota and secreted into the intestinal lumen in the presence of inflammation or pathogens in the epithelium (Artis et al., 2004; He et al., 2003; Vaishnava et al., 2008). RELMβ was recently shown to bind and kill Gram (-) bacteria by forming multimeric pores in the cell membrane, thus reducing penetration into the sterile inner layer of colonic mucus (Propheter et al., 2017). Different mechanisms have been identified for the control of RELMβ activation. One of them is through the activation of caudal-type homeobox 2 (CDX2) TF in the presence of bacteria or LPS (He et al., 2003; Wang et al., 2005). Interestingly, it has also been observed that the promoter of *Retnlb* has functional binding sites for HNF4α (Pine et al., 2018). Therefore, our results suggest that the induction of protection mechanisms in the intestine are probably directly regulated by HNF4α. However, and in contrary to our results, Chellapa *et al.* observed an overexpression of HNF4α P2 isoforms with parallel increases in RELMβ and inflammation symptoms during experimental colitis (Chellappa et al., 2016). Taking this into account, a more in-depth study of the precise network that controls HNF4α specific isoforms will be necessary. On the other hand, MUC3 is a transmembrane protein expressed apically in IECs and is part of the mucus layer that protects the epithelium. Due to its biological structure, MUC3 participates in cellular signaling processes through mediation from its internal cellular C-terminal region (Johansson and Hansson, 2016). It is known that some mucins can trigger signaling and response to invading pathogens through their intracellular tail (Kato et al., 2017). Likewise,

the increased expression of MUC3 in the colon has been reported as a defense mechanism against the binding of *E. coli* to the epithelium (Mack et al., 1999; Mack et al., 2003). Similarly to our observations, a reduced expression of *MUC3* gene transcripts is observed in the non-inflamed tissue of CD patients (Buisine et al., 1999). A decrease of *Muc3* despite an increase of *Retnlb* in the colon may indicate a predisposition to bacterial binding to the intestinal epithelium *Hnf4a*$^{\Delta IEC\text{-}ind}$ female mice.

In addition to *Retnlb* gene expression, *Hnf4a*$^{\Delta IEC\text{-}ind}$ males displayed increased of *Muc2* transcripts and protein levels in their ileum. MUC2 is the most abundant mucus-forming protein and is constitutively expressed in the intestine (Yamashita and Melo, 2018). Mucins are highly glycosylated proteins whose functions include the protection of the epithelium. It is known that these proteins can inhibit bacterial colonization through the antimicrobial activity of the carbohydrate chains that they present in their structure (Bravo and Correa, 1999; Genta et al., 1996; Teixeira et al., 2002). In this sense, Ferreira *et al.* demonstrated that aberrant expression of MUC2 was correlated with the elimination of *Helicobacter pylori* from the gastric mucosa filled with intestinal metaplasia lesions (Ferreira et al., 2006). On the other hand, it is known that infection with *Salmonella* Typhimurium in mice alters the expression and distribution of glycans, as well as reduces the content of mucin present in goblet cells. Likewise, *Muc2*$^{-/-}$ mice display an increased susceptibility to *Salmonella*. A specific analysis by immunostaining against MUC2 in mice infected with this bacterium revealed not only the presence of the secreted protein in the lumen after 3 days pi, but also an increase in goblet cells number in the cecal crypts when compared to non-infected mice (Zarepour et al., 2013). Our results show that the overexpression of *Muc2* in the ileum of male mice seems to be associated with the loss of HNF4α. Recently, a study of the avian *MUC2* gene promoter showed conserved binding sites for a group of TFs relevant to intestinal homeostasis, including CDX2, GATA binding protein 4 (GATA4), and HNF4α (Woodfint et al., 2017). Therefore, we could speculate that the *Muc2* gene may be down-regulated by HNF4α and its modulation could contribute positively to the protection of the barrier. The thickness and quality of the mucus are essential properties for the defense of the epithelium. In patients who develop IBD, the presence of a thinner layer of mucus was observed, which is a determining factor in the progression of the disease, colonization of the epithelium, and inflammation (Strugala et al., 2008). Although our results suggest that

Hnf4α$^{\Delta IEC\text{-}ind}$ male mice do not show an increase in MUC2 expression in colonic goblet cells, a preliminary analysis of the bacterial localization in the hydrogel showed a thicker and sterile inner mucus layer in these animals (Fig. 1, Annexes). This could suggest not only an increase in the synthesis of MUC2 but also in the rapidity of the release of the protein into the lumen. Therefore, it would be interesting to carry out a more detailed study regarding the ultrastructure of goblet cells, as well as the mechanisms that determine cell secretion in our model system.

Goblet cells are essential for the functionality of the epithelial barrier (Kim and Ho, 2010; Johansson and Hansson, 2016; Cornick et al., 2015). Interestingly, *Hnf4α*$^{\Delta IEC\text{-}ind}$ mice showed a high density of goblet cells in the ileal crypts. An increase in goblet cells frequency was previously described to occur in absence of intestinal HNF4α while the exact mechanisms involved in this phenomenon have not been fully elucidated (Babeu et al., 2009; Cattin et al., 2009). We also detected an increase in the goblet cells size along the crypt-villus axis for *Hnf4α*$^{\Delta IEC\text{-}ind}$ male mice. This phenomenon may be linked to an increase of the regulatory effect of HNF4α on secretory cells due to bacterial infection. Goblet cells are capable of permanently secreting low amounts of mucin to ensure the continuous formation of mucus. However, in the presence of a stimulus such as microbial products or cytokines, goblet cells can increase the production and rapid release of glycoproteins causing an expansion of the granules found within it expanding then their cellular volume (Lindén et al., 2008).

Finally, we were interested in investigating how the loss of HNF4α could be involved in the differentiation and maturation of goblet cells of the ileal epithelium. Interestingly, this analysis revealed that *Hnf4α*$^{\Delta IEC\text{-}ind}$ male mice express higher levels of *Math1* and *Spdef* transcripts. MATH1 is an early and key progenitor in differentiation to the secretory cell lineage (VanDussen and Samuelson, 2010). This gene is negatively regulated by the Notch signaling pathway (Kadesch, 2004). Observations carried out in intestinal *Math1*-deficient mice have revealed a composition of the epithelium mostly represented by absorptive cells (enterocytes) (Shroyer et al., 2007; van Es et al., 2010). On the other hand, SPDEF also has an essential role in the formation of goblet cells in the intestinal tissue (Noah et al., 2010). In this sense, Gregorieff *et al.* showed that *Spdef* $^{-/-}$ mice were severely impaired in Paneth and goblets cells maturation (Gregorieff et al., 2009). In addition, the Wnt/β-catenin pathway plays a predominant role in proliferation and differentiation of IECs (Fevr et al., 2007; Li et

al., 2019). It is also known that the bone morphogenic protein (BMP)/SMAD signaling pathway is related to the regulation of cell differentiation (Auclair et al., 2007; He et al., 2004). Interestingly, a recent study showed a close interaction of this pathway with HNF4α. HNF4α and the TF SMAD4 are capable of mutually activating and co-binding with regulatory elements that drive the cellular fate of enterocytes. However, impairment of this interaction results in a transcriptional restructuring, directing the differentiation towards the secretory cell lineage (Chen et al., 2019b). These results suggest that, especially in male mice, the loss of HNF4α induces a change in the renewal programs of the epithelium orchestrated in the intestinal crypts, which may constitute a defense mechanism closely linked to stem cells survival from a bacterial infection threat.

Throughout our study, we observed differential transcriptomic profiles between *Hnf4a*$^{\Delta IEC\text{-}ind}$ female and male mice. A potential explanation for this phenomenon may be related to the differential actions of hormones linked to sex. Estrogen is mainly synthesized in female reproductive tissues, although it is produced in less quantity by males. It can also be detected in other organs such as the liver, adrenal gland, and GI tract (Simpson, 2003). There are 3 types of estrogen, but the most relevant to growth, differentiation and other physiological processes of the body is 17β-estradiol (Iorga et al., 2017). Estrogen acts through an ER receptor that is composed of two classes, ERα and ERβ, which have different distributions and functions. The essential role of estrogen in maintaining the epithelial barrier has been previously elucidated. However, the dual role of this molecule has also been observed in inflammatory diseases such as IBD, due to its pro and anti-inflammatory actions (Nie et al., 2018). A clear example of the sex-dependent effect of estrogen was presented by Asai *et al.* in which they evidenced a negative modulation of the cytokine TNFα only in peripheral blood mononuclear cells of female patients after LPS induction (Asai et al., 2001). Sexual dimorphism in bacterial infections has been identified in human an animal model (Vázquez-Martínez et al., 2018). A study carried out by Textoris *et al*. evaluating the infection severity in mice with *Coxiella burnetti*, revealed that around 86% of the modulated genes were differentially expressed in male and female animals, evidencing a strong dependence on the sex. In this sense, the male mice showed an enrichment in gene clusters related to cell adhesion, signal transduction, defensins, etc, while female mice reported the activation of several genes connected to the circadian rhythm pathway and a new possible association with

the efficiency in the response of the pathogen elimination. In general, this phenomenon was mainly attributed to the presence of sex hormones (Textoris et al., 2010). Likewise, it is suggested that the differences linked to sex in terms of the induction of genes related to immunological functions in the gut, among other factors, it is influenced by the composition of the commensal microbiota (Kim et al., 2020). Elderman *et al*. from an analysis of the fecal microbiota, the expression of mRNA in colonic tissue and immune cells populations in C57B1/6OlaHsd and Balb/cOlaHsd mice, demonstrated a positive correlation between the enrichment of some bacterial species such as *Clostridium leptum* in female or *Faecalibacterium prausnitzii* and *Clostridium ramosum* in male, and the appearance of different gene expression profiles related to inflammatory responses, migration of leukocytes and proliferation and quantity of lymphocytes (Elderman et al., 2018).

5. CONCLUSIONS

In the present study, we wanted to clarify the involvement of HNF4α in the regulation of the intestinal epithelial barrier. The induced deletion of the *Hnf4a* gene in a murine model allowed us to elucidate possible mechanisms of the control that this TF exerts on IECs during the early events of enteric infection. Interestingly, our results confirm that HNF4α is capable of altering intestinal permeability by regulating expression of genes encoding tight junction proteins in IECs. However, against all odds, we observed that *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice were not more susceptible to *Salmonella typhimurium* infections. We found that *Hnf4a*$^{\Delta IEC\text{-}ind}$ mice presented an important variation in the number and size of goblet cells in the ileum after infection, and this more particularly in the intestinal crypt. Likewise, we identified that the loss of HNF4α impacted genes involved with the epithelial barrier protection and integrity, with a more marked effect in the *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice. All this indicates that, in mutant mice, an increase in the mucus layer and others factors that constitute the biochemical barrier probably compensate to ensure a protective effect of the mucosa in the absence of HNF4α. Taking these elements into account, we suggest that HNF4α could play an important adaptative role as a mediator of the intestinal epithelial barrier function in the presence of bacterial infections.

6. PERSPECTIVES

Although the decrease in HNF4α expression has been associated with risks of IBD, this NR may play a dual role in the functioning of the epithelium depending on the interactions that are established with other factors of the intestinal microenvironment. The immediate effect associated with an intestinal loss of HNF4α could be the trigger of a protective response. For example, increase of cell differentiation within the intestinal crypts towards the secretory cell lineage such as goblets cells could improve the epithelial barrier capacity against the attack of pathogens. In this sense, it will be necessary to analyze in greater depth how HNF4α is linked to the differentiation mechanisms that drive cell fate. This could be based on methodologies that evaluate loss of gene function in combination with other TFs. Besides, it would be interesting to characterize the morphology and maturation of goblet cells by electron microscopy in mutant and control mice, as well as to examine the mechanisms of mucin release in the mucus layer. Likewise, verifying the effect of HNF4α on Paneth cells and its involvement in the antimicrobial molecules synthesis would be a fundamental aspect to take into account for the maintenance of the biochemical barrier.

Furthermore, it will be important to validate transcriptomic changes observed for TJ and other identified barrier integrity regulators through western blot, IF, and ELISA. Due to the effect observed on paracellular permeability and ions transport, another important aspect in our study would be to determine by chromatin immunoprecipitations, luciferase reporter assays or EMSA, if there is a direct interaction between HNF4α and some of the genes identified as potential direct targets in this context.

Our study seems to indicate that there are sex-related differences of the impact HNF4α might have on the epithelial barrier. To validate these observations, it will be necessary to increase the number of animals to be used in the infection tests, as well as to evaluate other bacterial strains like *Citrobacter rodentium* to expand these findings.

Also, host-microbiota interactions are essential in the maintenance of intestinal homeostasis and it is known that the microbiota is capable of modulating the activity of HNF4α. Hence, an analysis of the microbiota composition and the induction of genes in the presence or not of HNF4α during intestinal dysbiosis would allow us to elucidate the activation of defense mechanisms in the epithelium.

Finally, with the aim to evaluate the direct effect of HNF4α on the epithelial barrier, it would be interesting pursuing *in vitro* study using two-dimensional monolayers obtained from the growth of intestinal 3D organoids (In et al., 2019; Roodsant et al., 2020). We have been able to establish jejunum and colon monolayers accordingly to the protocol described by Fernando *et al*., (Fig. 2, Annexes) (Fernando et al., 2017). However, we were unsuccessful in measuring significant transepithelial electrical resistance (TER) values normally associated with a functional epithelial barrier as previously reported (data not shown). The optimization of 2D monolayer cultures to assess epithelial barrier function will be of interest for this project. In addition, the use of other methodologies such as inside/out organoids (Co et al., 2019) will allow the pursuit of this aim.

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8. REFERENCES

Abraham, C., & Cho, J. H. (2009). Inflammatory Bowel Disease. New England Journal of Medicine, 361(21), 2066–2078. https://doi.org/10.1056/NEJMra0804647

Ahmad, R., Chaturvedi, R., Olivares-Villagómez, D., Habib, T., Asim, M., Shivesh, P., Polk, D. B., Wilson, K. T., Washington, M. K., Van Kaer, L., Dhawan, P., & Singh, A. B. (2014). Targeted colonic claudin-2 expression renders resistance to epithelial injury, induces immune suppression, and protects from colitis. *Mucosal Immunology*, *7*(6), 1340–1353. https://doi.org/10.1038/mi.2014.21

Ahn, S. H., Shah, Y. M., Inoue, J., Morimura, K., Kim, I., Yim, S. H., Lambert, G., Kurotani, R., Nagashima, K., Gonzalez, F. J., & Inoue, Y. (2008). Hepatocyte nuclear factor 4α in the intestinal epithelial cells protects against inflammatory bowel disease. *Inflammatory Bowel Diseases*, *14*(7), 908–920. https://doi.org/10.1002/ibd.20413

Al-Sadi, R., Ye, D., Said, H. M., & Ma, T. Y. (2010). IL-1β-induced increase in intestinal epithelial tight junction permeability is mediated by MEKK-1 activation of canonical NF-κB pathway. *American Journal of Pathology*, *177*(5), 2310–2322. https://doi.org/10.2353/ajpath.2010.100371

Alam, M. T., Amos, G. C. A., Murphy, A. R. J., Murch, S., Wellington, E. M. H., & Arasaradnam, R. P. (2020). Microbial imbalance in inflammatory bowel disease patients at different taxonomic levels. *Gut Pathogens*, *12*(1). https://doi.org/10.1186/s13099-019-0341-6

Albenberg, L., Esipova, T. V., Judge, C. P., Bittinger, K., Chen, J., Laughlin, A., Grunberg, S., Baldassano, R. N., Lewis, J. D., Li, H., Thom, S. R., Bushman, F. D., Vinogradov, S. A., & Wu, G. D. (2014). Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology*, *147*(5), 1055-1063.e8. https://doi.org/10.1053/j.gastro.2014.07.020

Alipour, M., Zaidi, D., Valcheva, R., Jovel, J., Martínez, I., Sergi, C., Walter, J., Mason, A. L., Ka-Shu Wong, G., Dieleman, L. A., Carroll, M. W., Huynh, H. Q., & Winea, E. (2016). Mucosal barrier depletion and loss of bacterial diversity are primary abnormalities in paediatric ulcerative colitis. *Journal of Crohn's and Colitis*, *10*(4), 462–471. https://doi.org/10.1093/ecco-jcc/jjv223

Allaire, J. M., Crowley, S. M., Law, H. T., Chang, S. Y., Ko, H. J., & Vallance, B. A. (2018). The Intestinal Epithelium: Central Coordinator of Mucosal Immunity. In *Trends in Immunology* (Vol. 39, Issue 9, pp. 677–696). Elsevier Ltd. https://doi.org/10.1016/j.it.2018.04.002

Allam-Ndoul, B., Castonguay-Paradis, S., & Veilleux, A. (2020). Gut microbiota and intestinal trans-epithelial permeability. In *International Journal of Molecular Sciences* (Vol. 21, Issue 17, pp. 1–14). MDPI AG. https://doi.org/10.3390/ijms21176402

Allen, A., Hutton, D. A., & Pearson, J. P. (1998). The MUC2 gene product: A human intestinal mucin. *International Journal of Biochemistry and Cell Biology*, *30*(7), 797–801. https://doi.org/10.1016/S1357-2725(98)00028-4

Amasheh, S., Meiri, N., Gitter, A. H., Schöneberg, T., Mankertz, J., Schulzke, J. D., & Fromm, M. (2002). Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. *Journal of Cell Science*, *115*(24), 4969–4976. https://doi.org/10.1242/jcs.00165

Amasheh, S., Milatz, S., Krug, S. M., Markov, A. G., Günzel, D., Amasheh, M., & Fromm, M. (2009). Tight junction proteins as channel formers and barrier builders: Claudin-2, -

5, and -8. *Annals of the New York Academy of Sciences*, *1165*, 211–219. https://doi.org/10.1111/j.1749-6632.2009.04439.x

Anderson, C. A., Boucher, G., Lees, C. W., Franke, A., D'Amato, M., Taylor, K. D., Lee, J. C., Goyette, P., Imielinski, M., Latiano, A., Lagacé, C., Scott, R., Amininejad, L., Bumpstead, S., Baidoo, L., Baldassano, R. N., Barclay, M., Bayless, T. M., Brand, S., … Rioux, J. D. (2011). Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. In *Nature Genetics* (Vol. 43, Issue 3, pp. 246–252). Nature Publishing Group. https://doi.org/10.1038/ng.764

Antoni, L., Nuding, S., Wehkamp, J., & Stange, E. F. (2014). Intestinal barrier in inflammatory bowel disease. *World Journal of Gastroenterology*, *20*(5), 1165–1179. https://doi.org/10.3748/wjg.v20.i5.1165

Arnold, J. W., Niesel, D. W., Annable, C. R., Hess, C. B., Asuncion, M., Cho, Y. J., Peterson, J. W., & Klimpel, G. R. (1993). Tumor necrosis factor-α mediates the early pathology in Salmonella infection of the gastrointestinal tract. *Microbial Pathogenesis*, *14*(3), 217–227. https://doi.org/10.1006/mpat.1993.1021

Artis, D., Wang, M. L., Keilbaugh, S. A., He, W., Brenes, M., Swain, G. P., Knight, P. A., Donaldson, D. D., Lazar, M. A., Miller, H. R. P., Schad, G. A., Scott, P., & Wu, G. D. (2004). RELMβ/FIZZ2 is a goblet cell-specific immune-effector molecule in the gastrointestinal tract. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(37), 13596–13600. https://doi.org/10.1073/pnas.0404034101

Asai, K., Hiki, N., Mimura, Y., Ogawa, T., Unou, K., & Kaminishi, M. (2001). Gender differences in cytokine secretion by human peripheral blood mononuclear cells: Role of estrogen in modulating LPS-induced cytokine secretion in an ex vivo septic model. *Shock*, *16*(5), 340–343. https://doi.org/10.1097/00024382-200116050-00003

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K., Kim, S., Fritz, J. V., Wilmes, P., Ueha, S., Matsushima, K., Ohno, H., Olle, B., Sakaguchi, S., Taniguchi, T., … Honda, K. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*, *500*(7461), 232–236. https://doi.org/10.1038/nature12331

Atuma, C., Strugala, V., Allen, A., & Holm, L. (2001). The adherent gastrointestinal mucus gel layer: Thickness and physical state in vivo. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *280*(5 43-5). https://doi.org/10.1152/ajpgi.2001.280.5.g922

Auclair, B. A., Benoit, Y. D., Rivard, N., Mishina, Y., & Perreault, N. (2007). Bone Morphogenetic Protein Signaling Is Essential for Terminal Differentiation of the Intestinal Secretory Cell Lineage. *Gastroenterology*, *133*(3), 887–896. https://doi.org/10.1053/j.gastro.2007.06.066

Babeu, J. P., & Boudreau, F. (2014). Hepatocyte nuclear factor 4-alpha involvement in liver and intestinal inflammatory networks. *World Journal of Gastroenterology*, *20*(1), 22–30. https://doi.org/10.3748/wjg.v20.i1.22

Babeu, J. P., Darsigny, M., Lussier, C. R., & Boudreau, F. (2009). Hepatocyte nuclear factor 4α contributes to an intestinal epithelial phenotype in vitro and plays a partial role in mouse intestinal epithelium differentiation. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *297*(1). https://doi.org/10.1152/ajpgi.90690.2008

Barker, N. (2014). Adult intestinal stem cells: Critical drivers of epithelial homeostasis and regeneration. *Nature Reviews Molecular Cell Biology*, *15*(1), 19–33.

https://doi.org/10.1038/nrm3721

Barr, J. J., Auro, R., Furlan, M., Whiteson, K. L., Erb, M. L., Pogliano, J., Stotland, A., Wolkowicz, R., Cutting, A. S., Doran, K. S., Salamon, P., Youle, M., & Rohwer, F. (2013). Bacteriophage adhering to mucus provide a non-host-derived immunity. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(26), 10771–10776. https://doi.org/10.1073/pnas.1305923110

Barrett, J. C., Hansoul, S., Nicolae, D. L., Cho, J. H., Duerr, R. H., Rioux, J. D., Brant, S. R., Silverberg, M. S., Taylor, K. D., Barmada, M. M., Bitton, A., Dassopoulos, T., Datta, L. W., Green, T., Griffiths, A. M., Kistner, E. O., Murtha, M. T., Regueiro, M. D., Rotter, J. I., … Daly, M. J. (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nature Genetics*, *40*(8), 955–962. https://doi.org/10.1038/ng.175

Barrett, J. C., Lee, J. C., Lees, C. W., Prescott, N. J., Anderson, C. A., Phillips, A., Wesley, E., Parnell, K., Zhang, H., Drummond, H., Nimmo, E. R., Massey, D., Blaszczyk, K., Elliott, T., Cotterill, L., Dallal, H., Lobo, A. J., Mowat, C., Sanderson, J. D., … Strachan, D. P. (2009). Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nature Genetics*, *41*(12), 1330–1334. https://doi.org/10.1038/ng.483

Battle, M. A., Konopka, G., Parviz, F., Gaggl, A. L., Yang, C., Sladek, F. M., & Duncan, S. A. (2006). Hepatocyte nuclear factor 4α orchestrates expression of cell adhesion proteins during the epithelial transformation of the developing liver. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(22), 8419–8424. https://doi.org/10.1073/pnas.0600246103

Bel, S., Pendse, M., Wang, Y., Li, Y., Ruhn, K. A., Hassell, B., Leal, T., Winter, S. E., Xavier, R. J., & Hooper, L. V. (2017). Paneth cells secrete lysozyme via secretory autophagy during bacterial infection of the intestine. *Science*, *357*(6355), 1047–1052. https://doi.org/10.1126/science.aal4677

Bell, A., Brunt, J., Crost, E., Vaux, L., Nepravishta, R., Owen, C. D., Latousakis, D., Xiao, A., Li, W., Chen, X., Walsh, M. A., Claesen, J., Angulo, J., Thomas, G. H., & Juge, N. (2019). Elucidation of a sialic acid metabolism pathway in mucus-foraging Ruminococcus gnavus unravels mechanisms of bacterial adaptation to the gut. *Nature Microbiology*, *4*(12), 2393–2404. https://doi.org/10.1038/s41564-019-0590-7

Bergstrom, K. S. B., Kissoon-Singh, V., Gibson, D. L., Ma, C., Montero, M., Sham, H. P., Ryz, N., Huang, T., Velcich, A., Finlay, B. B., Chadee, K., & Vallance, B. A. (2010). Muc2 protects against lethal infectious colitis by disassociating pathogenic and commensal bacteria from the colonic mucosa. *PLoS Pathogens*, *6*(5). https://doi.org/10.1371/journal.ppat.1000902

Bertocchi, C., Vaman Rao, M., & Zaidel-Bar, R. (2012). Regulation of Adherens Junction Dynamics by Phosphorylation Switches. *Journal of Signal Transduction*, *2012*, 1–14. https://doi.org/10.1155/2012/125295

Bevins, C. L., & Salzman, N. H. (2011). Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. In *Nature Reviews Microbiology* (Vol. 9, Issue 5, pp. 356–368). https://doi.org/10.1038/nrmicro2546

Bhinder, G., Stahl, M., Sham, H. P., Crowley, S. M., Morampudi, V., Dalwadi, U., Ma, C., Jacobson, K., & Vallance, B. A. (2014). Intestinal epithelium-specific MyD88 signaling impacts host susceptibility to infectious colitis by promoting protective goblet cell and antimicrobial responses. *Infection and Immunity*, *82*(9), 3753–3763.

https://doi.org/10.1128/IAI.02045-14

Bien, J., Palagani, V., & Bozko, P. (2013). The intestinal microbiota dysbiosis and Clostridium difficile infection: Is there a relationship with inflammatory bowel disease? In *Therapeutic Advances in Gastroenterology* (Vol. 6, Issue 1, pp. 53–68). https://doi.org/10.1177/1756283X12454590

Birchenough, G. M. H., Johansson, M. E. V., Gustafsson, J. K., Bergström, J. H., & Hansson, G. C. (2015). New developments in goblet cell mucus secretion and function. In *Mucosal Immunology* (Vol. 8, Issue 4, pp. 712–719). Nature Publishing Group. https://doi.org/10.1038/mi.2015.32

Bischoff, S. C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J. D., Serino, M., Tilg, H., Watson, A., & Wells, J. M. (2014). Intestinal permeability - a new target for disease prevention and therapy. In *BMC Gastroenterology* (Vol. 14, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12876-014-0189-7

Blair, S. A., Kane, S. V., Clayburgh, D. R., & Turner, J. R. (2006). Epithelial myosin light chain kinase expression and activity are upregulated in inflammatory bowel disease. *Laboratory Investigation*, *86*(2), 191–201. https://doi.org/10.1038/labinvest.3700373

Brandtzaeg, P. (2013). Secretory IgA: Designed for anti-microbial defense. *Frontiers in Immunology*, *4*(AUG). https://doi.org/10.3389/fimmu.2013.00222

Bravo, J. C., & Correa, P. (1999). Sulphomucins favour adhesion of Helicobacter pylori to metaplastic gastric mucosa. *Journal of Clinical Pathology*, *52*(2), 137–140. https://doi.org/10.1136/jcp.52.2.137

Bridgham, J. T., Eick, G. N., Larroux, C., Deshpande, K., Harms, M. J., Gauthier, M. E. A., Ortlund, E. A., Degnan, B. M., & Thornton, J. W. (2010). Protein evolution by molecular tinkering: Diversification of the nuclear receptor superfamily from a ligand-dependent ancestor. *PLoS Biology*, *8*(10). https://doi.org/10.1371/journal.pbio.1000497

Brown, D. E., Libby, S. J., Moreland, S. M., McCoy, M. W., Brabb, T., Stepanek, A., Fang, F. C., & Detweiler, C. S. (2013). Salmonella enterica Causes More Severe Inflammatory Disease in C57/BL6 Nramp1G169 Mice Than Sv129S6 Mice. *Veterinary Pathology*, *50*(5), 867–876. https://doi.org/10.1177/0300985813478213

Broz, P., Ohlson, M. B., & Monack, D. M. (2012). Innate immune response to Salmonella typhimurium, a model enteric pathogen. *Gut Microbes*, *3*(2), 62–70. https://doi.org/10.4161/gmic.19141

Bruewer, M., Utech, M., Ivanov, A. I., Hopkins, A. M., Parkos, C. A., & Nusrat, A. (2005). Interferon-γ induces internalization of epithelial tight junction proteins via a macropinocytosis-like process. *The FASEB Journal*, *19*(8), 923–933. https://doi.org/10.1096/fj.04-3260com

Bücker, R., Schumann, M., Amasheh, S., & Schulzke, J. D. (2010). Claudins in Intestinal Function and Disease. In *Current Topics in Membranes* (Vol. 65, Issue C). https://doi.org/10.1016/S1063-5823(10)65009-0

Buisine, M. P., Desreumaux, P., Leteurtre, E., Copin, M. C., Colombel, J. F., Porchet, N., & Aubert, J. P. (2001). Mucin gene expression in intestinal epithelial cells in Crohn's disease. *Gut*, *49*(4), 544–551. https://doi.org/10.1136/gut.49.4.544

Buisine, Marie Pierre, Desreumaux, P., Debailleul, V., Gambiez, L., Geboes, K., Ectors, N., Delescaut, M. P., Degand, P., Aubert, J. P., Colombel, J. F., & Porchet, N. (1999). Abnormalities in mucin gene expression in Crohn's disease. *Inflammatory Bowel Diseases*, *5*(1), 24–32. https://doi.org/10.1097/00054725-199902000-00004

Buschmann, M. M., Shen, L., Rajapakse, H., Raleigh, D. R., Wang, Y., Wang, Y., Lingaraju,

A., Zha, J., Abbott, E., McAuley, E. M., Breskin, L. A., Wu, L., Anderson, K., Turner, J. R., & Weber, C. R. (2013). Occludin OCEL-domain interactions are required for maintenance and regulation of the tight junction barrier to macromolecular flux. *Molecular Biology of the Cell*, *24*(19), 3056–3068. https://doi.org/10.1091/mbc.E12-09-0688

Byndloss, M. X., Olsan, E. E., Rivera-Chávez, F., Tiffany, C. R., Cevallos, S. A., Lokken, K. L., Torres, T. P., Byndloss, A. J., Faber, F., Gao, Y., Litvak, Y., Lopez, C. A., Xu, G., Napoli, E., Giulivi, C., Tsolis, R. M., Revzin, A., Lebrilla, C. B., & Bäumler, A. J. (2017). Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. *Science*, *357*(6351), 570–575. https://doi.org/10.1126/science.aam9949

Capaldo, C. T., & Nusrat, A. (2015). Claudin switching: Physiological plasticity of the Tight Junction. In *Seminars in Cell and Developmental Biology* (Vol. 42, pp. 22–29). Academic Press. https://doi.org/10.1016/j.semcdb.2015.04.003

Castillo, P. A., Nonnecke, E. B., Ossorio, D. T., Tran, M. T. N., Goley, S. M., Lönnerdal, B., Underwood, M. A., & Bevins, C. L. (2019). An Experimental Approach to Rigorously Assess Paneth Cell α-Defensin (Defa) mRNA Expression in C57BL/6 Mice. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-49471-9

Cattin, A.-L., Le Beyec, J., Barreau, F., Saint-Just, S., Houllier, A., Gonzalez, F. J., Robine, S., Pinçon-Raymond, M., Cardot, P., Lacasa, M., & Ribeiro, A. (2009). Hepatocyte Nuclear Factor 4α, a Key Factor for Homeostasis, Cell Architecture, and Barrier Function of the Adult Intestinal Epithelium. *Molecular and Cellular Biology*, *29*(23), 6294–6308. https://doi.org/10.1128/mcb.00939-09

Cereijido, M., Contreras, R. G., Flores-Benítez, D., Flores-Maldonado, C., Larre, I., Ruiz, A., & Shoshani, L. (2007). New Diseases Derived or Associated with the Tight Junction. In *Archives of Medical Research* (Vol. 38, Issue 5, pp. 465–478). Elsevier Inc. https://doi.org/10.1016/j.arcmed.2007.02.003

Cerutti, A. (2008). The regulation of IgA class switching. In *Nature Reviews Immunology* (Vol. 8, Issue 6, pp. 421–434). https://doi.org/10.1038/nri2322

Chang, J., Leong, R. W., Wasinger, V. C., Ip, M., Yang, M., & Phan, T. G. (2017). Impaired Intestinal Permeability Contributes to Ongoing Bowel Symptoms in Patients With Inflammatory Bowel Disease and Mucosal Healing. *Gastroenterology*, *153*(3), 723-731.e1. https://doi.org/10.1053/j.gastro.2017.05.056

Chassaing, B., & Darfeuillemichaud, A. (2011). The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. *Gastroenterology*, *140*(6), 1720-1728.e3. https://doi.org/10.1053/j.gastro.2011.01.054

Chellappa, K., Deol, P., Evans, J. R., Vuong, L. M., Chen, G., Briançon, N., Bolotin, E., Lytle, C., Nair, M. G., & Sladek, F. M. (2016). Opposing roles of nuclear receptor HNF4α isoforms in colitis and colitis-associated colon cancer. *ELife*, *5*(MAY2016). https://doi.org/10.7554/eLife.10903

Chen, L., Toke, N. H., Luo, S., Vasoya, R. P., Aita, R., Parthasarathy, A., Tsai, Y. H., Spence, J. R., & Verzi, M. P. (2019). HNF4 factors control chromatin accessibility and are redundantly required for maturation of the fetal intestine. *Development (Cambridge)*, *146*(19). https://doi.org/10.1242/dev.179432

Chen, L., Toke, N. H., Luo, S., Vasoya, R. P., Fullem, R. L., Parthasarathy, A., Perekatt, A. O., & Verzi, M. P. (2019). A reinforcing HNF4–SMAD4 feed-forward module stabilizes enterocyte identity. In *Nature Genetics* (Vol. 51, Issue 5, pp. 777–785). Nature Publishing Group. https://doi.org/10.1038/s41588-019-0384-0

Chen, W. S., Manova, K., Weinstein, D. C., Duncan, S. A., Plump, A. S., Prezioso, V. R., Bachvarova, R. F., & Darnell, J. E. (1994). Disruption of the HNF-4 gene, expressed in visceral endoderm, leads to cell death in embryonic ectoderm and impaired gastrulation of mouse embryos. *Genes and Development*, *8*(20), 2466–2477. https://doi.org/10.1101/gad.8.20.2466

Cheng, R. A., Eade, C. R., & Wiedmann, M. (2019). Embracing diversity: Differences in virulence mechanisms, disease severity, and host adaptations contribute to the success of nontyphoidal salmonellaas a foodborne pathogen. *Frontiers in Microbiology*, *10*(JUN). https://doi.org/10.3389/fmicb.2019.01368

Chu, H., Pazgier, M., Jung, G., Nuccio, S. P., Castillo, P. A., De Jong, M. F., Winter, M. G., Winter, S. E., Wehkamp, J., Shen, B., Salzman, N. H., Underwood, M. A., Tsolis, R. M., Young, G. M., Lu, W., Lehrer, R. I., Bäumler, A. J., & Bevins, C. L. (2012). Human α-defensin 6 promotes mucosal innate immunity through self-assembled peptide nanonets. *Science*, *337*(6093), 477–481. https://doi.org/10.1126/science.1218831

Clayburgh, D. R., Rosen, S., Witkowski, E. D., Wang, F., Blair, S., Dudek, S., Garcia, J. G. N., Alverdy, J. C., & Turner, J. R. (2004). A differentiation-dependent splice variant of myosin light chain kinase, MLCK1, regulates epithelial tight junction permeability. *Journal of Biological Chemistry*, *279*(53), 55506–55513. https://doi.org/10.1074/jbc.M408822200

Co, J. Y., Margalef-Català, M., Li, X., Mah, A. T., Kuo, C. J., Monack, D. M., & Amieva, M. R. (2019). Controlling Epithelial Polarity: A Human Enteroid Model for Host-Pathogen Interactions. *Cell Reports*, *26*(9), 2509-2520.e4. https://doi.org/10.1016/j.celrep.2019.01.108

Consortium, W. T. C. C., Burton, P. R., Donnelly, P., Cardon, L. R., Clayton, D. G., Todd, J. A., Ouwehand, W. H., Craddock, N., Tobin, M. D., Samani, N. J., Nutland, S., Stevens, H. E., Walker, N. M., McCarthy, M. I., Newport, M., Sirugo, G., Kwiatkowski, D. P., Deloukas, P., Leung, H.-T., … Worthington, J. (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*, *447*(7145), 661–678. http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=17554300&retmode=ref&cmd=prlinks

Cording, J., Berg, J., Käding, N., Bellmann, C., Tscheik, C., Westphal, J. K., Milatz, S., Günzel, D., Wolburg, H., Piontek, J., Huber, O., & Blasig, I. E. (2013). In tight junctions, claudins regulate the interactions between occludin, tricellulin and marvelD3, which, inversely, modulate claudin oligomerization. *Journal of Cell Science*, *126*(2), 554–564. https://doi.org/10.1242/jcs.114306

Cornick, S., Tawiah, A., & Chadee, K. (2015). Roles and regulation of the mucus barrier in the gut. In *Tissue Barriers* (Vol. 3, Issue 1). Taylor and Francis Inc. https://doi.org/10.4161/21688370.2014.982426

Crowley, S. M., Han, X., Allaire, J. M., Stahl, M., Rauch, I., Knodler, L. A., & Vallance, B. A. (2020). Intestinal restriction of Salmonella Typhimurium requires caspase-1 and caspase-11 epithelial intrinsic inflammasomes. *PLoS Pathogens*, *16*(4). https://doi.org/10.1371/journal.ppat.1008498

D'Argenio, V., Casaburi, G., Precone, V., Pagliuca, C., Colicchio, R., Sarnataro, D., Discepolo, V., Kim, S. M., Russo, I., Del Vecchio Blanco, G., Horner, D. S., Chiara, M., Pesole, G., Salvatore, P., Monteleone, G., Ciacci, C., Caporaso, G. J., Jabrì, B., Salvatore, F., & Sacchetti, L. (2016). Metagenomics reveals dysbiosis and a potentially

pathogenic N. flavescens strain in duodenum of adult celiac patients. *American Journal of Gastroenterology*, *111*(6), 879–890. https://doi.org/10.1038/ajg.2016.95

Darfeuille-Michaud, A. (2002). Adherent-invasive Escherichia coli: A putative new E. coli pathotype associated with Crohn's disease. In *International Journal of Medical Microbiology* (Vol. 292, Issues 3–4, pp. 185–193). Elsevier GmbH. https://doi.org/10.1078/1438-4221-00201

Darsigny, M., Babeu, J. P., Dupuis, A. A., Furth, E. E., Seidman, E. G., Lévy, É., Verdu, E. F., Gendron, F. P., & Boudreau, F. (2009). Loss of hepatocyte-nuclear-factor-4α affects colonic ion transport and causes chronic inflammation resembling inflammatory bowel disease in mice. *PLoS ONE*, *4*(10). https://doi.org/10.1371/journal.pone.0007609

Darsigny, M., Babeu, J. P., Seidman, E. G., Gendron, F. P., Levy, E., Carrier, J., Perreault, N., & Boudreau, F. (2010). Hepatocyte nuclear factor-4α promotes gut neoplasia in mice and protects against the production of reactive oxygen species. *Cancer Research*, *70*(22), 9423–9433. https://doi.org/10.1158/0008-5472.CAN-10-1697

Das, P., Goswami, P., Das, T. K., Nag, T., Sreenivas, V., Ahuja, V., Panda, S. K., Gupta, S. D., & Makharia, G. K. (2012). Comparative tight junction protein expressions in colonic Crohn's disease, ulcerative colitis, and tuberculosis: A new perspective. *Virchows Archiv*, *460*(3), 261–270. https://doi.org/10.1007/s00428-012-1195-1

David, J. M., & Rajasekaran, A. K. (2012). Dishonorable discharge: The oncogenic roles of cleaved e-cadherin fragments. In *Cancer Research* (Vol. 72, Issue 12, pp. 2917–2923). https://doi.org/10.1158/0008-5472.CAN-11-3498

Davison, J. M., Lickwar, C. R., Song, L., Breton, G., Crawford, G. E., & Rawls, J. F. (2017). Microbiota regulate intestinal epithelial gene expression by suppressing the transcription factor Hepatocyte nuclear factor 4 alpha. *Genome Research*, *27*(7), 1195–1206. https://doi.org/10.1101/gr.220111.116

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G., & Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(33), 14691–14696. https://doi.org/10.1073/pnas.1005963107

De Hertogh, G., Aerssens, J., Geboes, K. P., & Geboes, K. P. (2008). Evidence for the involvement of infectious agents in the pathogenesis of Crohn's disease. In *World Journal of Gastroenterology* (Vol. 14, Issue 6, pp. 845–852). https://doi.org/10.3748/wjg.14.845

De Lange, K. M., Moutsianas, L., Lee, J. C., Lamb, C. A., Luo, Y., Kennedy, N. A., Jostins, L., Rice, D. L., Gutierrez-Achury, J., Ji, S. G., Heap, G., Nimmo, E. R., Edwards, C., Henderson, P., Mowat, C., Sanderson, J., Satsangi, J., Simmons, A., Wilson, D. C., … Barrett, J. C. (2017). Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease. *Nature Genetics*, *49*(2), 256–261. https://doi.org/10.1038/ng.3760

De Souza, H. S. P., & Fiocchi, C. (2016). Immunopathogenesis of IBD: Current state of the art. In *Nature Reviews Gastroenterology and Hepatology* (Vol. 13, Issue 1, pp. 13–27). Nature Publishing Group. https://doi.org/10.1038/nrgastro.2015.186

Dekker, J., Rossen, J. W. A., Büller, H. A., & Einerhand, A. W. C. (2002). The MUC family: An obituary. In *Trends in Biochemical Sciences* (Vol. 27, Issue 3, pp. 126–131). https://doi.org/10.1016/S0968-0004(01)02052-7

Desilets, M., Deng, X., Rao, C., Ensminger, A. W., Krause, D. O., Sherman, P. M., & Gray-

Owen, S. D. (2016). Genome-based Definition of an Inflammatory Bowel Disease-associated Adherent-Invasive Escherichia coli Pathovar. *Inflammatory Bowel Diseases*, *22*(1), 1–12. https://doi.org/10.1097/MIB.0000000000000574

DH, K., KG, M., KA, K., JK, G., KM, K., DA, H., MJ, M., & RD, N. (2018). Goblet cell associated antigen passages are inhibited during Salmonella typhimurium infection to prevent pathogen dissemination and limit responses to dietary antigens. *Mucosal Immunology*, *11*(4), 1103–1113. http://www.nature.com/articles/doi:10.1038/s41385-018-0007-6

Dhanisha, S. S., Guruvayoorappan, C., Drishya, S., & Abeesh, P. (2018). Mucins: Structural diversity, biosynthesis, its role in pathogenesis and as possible therapeutic targets. In *Critical Reviews in Oncology/Hematology* (Vol. 122, pp. 98–122). Elsevier Ireland Ltd. https://doi.org/10.1016/j.critrevonc.2017.12.006

Dinarello, C. A., & Fantuzzi, G. (2003). Interleukin-18 and host defense against infection. *Journal of Infectious Diseases*, *187*(SUPPL. 2). https://doi.org/10.1086/374751

Ding, L., Lu, Z., Foreman, O., Tatum, R., Lu, Q., Renegar, R., Cao, J., & Chen, Y. (2012). Inflammation and disruption of the mucosal architecture in claudin-7-deficient mice. *Gastroenterology*, *142*(2), 305–315. https://doi.org/10.1053/j.gastro.2011.10.025

Donocoff, R. S., Teteloshvili, N., Chung, H., Shoulson, R., & Creusot, R. J. (2020). Optimization of tamoxifen-induced Cre activity and its effect on immune cell populations. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-72179-0

Dotti, I., Mora-Buch, R., Ferrer-Picón, E., Planell, N., Jung, P., Masamunt, M. C., Leal, R. F., De Carpi, J. M., Llach, J., Ordás, I., Batlle, E., Panés, J., & Salas, A. (2017). Alterations in the epithelial stem cell compartment could contribute to permanent changes in the mucosa of patients with ulcerative colitis. *Gut*, *66*(12). https://doi.org/10.1136/gutjnl-2016-312609

Drewes, T., Senkel, S., Holewa, B., & Ryffel, G. U. (1996). Human hepatocyte nuclear factor 4 isoforms are encoded by distinct and differentially expressed genes. *Molecular and Cellular Biology*, *16*(3), 925–931. https://doi.org/10.1128/mcb.16.3.925

Du, J., Chen, Y., Shi, Y., Liu, T., Cao, Y., Tang, Y., Ge, X., Nie, H., Zheng, C., & Li, Y. C. (2015). 1,25-Dihydroxyvitamin D protects intestinal epithelial barrier by regulating the myosin light chain kinase signaling pathway. *Inflammatory Bowel Diseases*, *21*(11), 2495–2506. https://doi.org/10.1097/MIB.0000000000000526

Dupont, A., Heinbockel, L., Brandenburg, K., & Hornef, M. W. (2015). Antimicrobial peptides and the enteric mucus layer act in concert to protect the intestinal mucosa. *Gut Microbes*, *5*(6), 761–765. https://doi.org/10.4161/19490976.2014.972238

Eck, A., De Groot, E. F. J., De Meij, T. G. J., Welling, M., Savelkoul, P. H. M., & Budding, A. E. (2017). Robust microbiota-based diagnostics for inflammatory bowel disease. *Journal of Clinical Microbiology*, *55*(6), 1720–1732. https://doi.org/10.1128/JCM.00162-17

El Marjou, F., Janssen, K. P., Chang, B. H. J., Li, M., Hindie, V., Chan, L., Louvard, D., Chambon, P., Metzger, D., & Robine, S. (2004). Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. *Genesis*, *39*(3), 186–193. https://doi.org/10.1002/gene.20042

Elderman, M., Hugenholtz, F., Belzer, C., Boekschoten, M., van Beek, A., de Haan, B., Savelkoul, H., de Vos, P., & Faas, M. (2018). Sex and strain dependent differences in mucosal immunology and microbiota composition in mice. *Biology of Sex Differences*, *9*(1), 26. https://doi.org/10.1186/s13293-018-0186-6

Elphick, D. A., & Mahida, Y. R. (2005). Paneth cells: Their role in innate immunity and inflammatory disease. In *Gut* (Vol. 54, Issue 12, pp. 1802–1809). https://doi.org/10.1136/gut.2005.068601

Ermund, A., Schütte, A., Johansson, M. E. V., Gustafsson, J. K., & Hansson, G. C. (2013). Studies of mucus in mouse stomach, small intestine, and colon. I. Gastrointestinal mucus layers have different properties depending on location as well as over the Peyer's patches. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *305*(5). https://doi.org/10.1152/ajpgi.00046.2013

Faderl, M., Noti, M., Corazza, N., & Mueller, C. (2015). Keeping bugs in check: The mucus layer as a critical component in maintaining intestinal homeostasis. In *IUBMB Life* (Vol. 67, Issue 4, pp. 275–285). Blackwell Publishing Ltd. https://doi.org/10.1002/iub.1374

Farkas, A. E., Hilgarth, R. S., Capaldo, C. T., Gerner-Smidt, C., Powell, D. R., Vertino, P. M., Koval, M., Parkos, C. A., & Nusrat, A. (2015). HNF4α Regulates Claudin-7 Protein Expression during Intestinal Epithelial Differentiation. *American Journal of Pathology*, *185*(8), 2206–2218. https://doi.org/10.1016/j.ajpath.2015.04.023

Fernando, E. H., Dicay, M., Stahl, M., Gordon, M. H., Vegso, A., Baggio, C., Alston, L., Lopes, F., Baker, K., Hirota, S., McKay, D. M., Vallance, B., & MacNaughton, W. K. (2017). A simple, cost-effective method for generating murine colonic 3D enteroids and 2D monolayers for studies of primary epithelial cell function. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *313*(5), G467–G475. https://doi.org/10.1152/ajpgi.00152.2017

Ferreira, B., Marcos, N. T., David, L., Nakayama, J., & Reis, C. A. (2006). Terminal α1,4-linked N-acetylglucosamine in Helicobacter pylori-associated intestinal metaplasia of the human stomach and gastric carcinoma cell lines. *Journal of Histochemistry and Cytochemistry*, *54*(5), 585–591. https://doi.org/10.1369/jhc.5A6836.2006

Fevr, T., Robine, S., Louvard, D., & Huelsken, J. (2007). Wnt/β-Catenin Is Essential for Intestinal Homeostasis and Maintenance of Intestinal Stem Cells. *Molecular and Cellular Biology*, *27*(21), 7551–7559. https://doi.org/10.1128/mcb.01034-07

Fihn, B. M., Sjoqvist, A., & Jodal, M. (2000). Permeability of the rat small intestinal epithelium along the villus-crypt axis: Effects of glucose transport. *Gastroenterology*, *119*(4), 1029–1036. https://doi.org/10.1053/gast.2000.18148

Findley, M. K., & Koval, M. (2009). Regulation and roles for claudin-family tight junction proteins. In *IUBMB Life* (Vol. 61, Issue 4, pp. 431–437). https://doi.org/10.1002/iub.175

Franke, A., McGovern, D. P. B., Barrett, J. C., Wang, K., Radford-Smith, G. L., Ahmad, T., Lees, C. W., Balschun, T., Lee, J., Roberts, R., Anderson, C. A., Bis, J. C., Bumpstead, S., Ellinghaus, D., Festen, E. M., Georges, M., Green, T., Haritunians, T., Jostins, L., … Parkes, M. (2010). Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nature Genetics*, *42*(12), 1118–1125. https://doi.org/10.1038/ng.717

Franzosa, E. A., Sirota-Madi, A., Avila-Pacheco, J., Fornelos, N., Haiser, H. J., Reinker, S., Vatanen, T., Hall, A. B., Mallick, H., McIver, L. J., Sauk, J. S., Wilson, R. G., Stevens, B. W., Scott, J. M., Pierce, K., Deik, A. A., Bullock, K., Imhann, F., Porter, J. A., … Xavier, R. J. (2019). Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nature Microbiology*, *4*(2), 293–305. https://doi.org/10.1038/s41564-018-0306-4

Fu, J., Wei, B., Wen, T., Johansson, M. E. V., Liu, X., Bradford, E., Thomsson, K. A., McGee, S., Mansour, L., Tong, M., McDaniel, J. M., Sferra, T. J., Turner, J. R., Chen,

H., Hansson, G. C., Braun, J., & Xia, L. (2011). Loss of intestinal core 1-derived O-glycans causes spontaneous colitis in mice. *Journal of Clinical Investigation*, *121*(4), 1657–1666. https://doi.org/10.1172/JCI45538

Fujita, H., Sugimoto, K., Inatomi, S., Maeda, T., Osanai, M., Uchiyama, Y., Yamamoto, Y., Wada, T., Kojima, T., Yokozaki, H., Yamashita, T., Kato, S., Sawada, N., & Chiba, H. (2008). Tight junction proteins claudin-2 and -12 are critical for vitamin D-dependent Ca2+ absorption between enterocytes. *Molecular Biology of the Cell*, *19*(5), 1912–1921. https://doi.org/10.1091/mbc.E07-09-0973

Furter, M., Sellin, M. E., Hansson, G. C., & Hardt, W. D. (2019). Mucus Architecture and Near-Surface Swimming Affect Distinct Salmonella Typhimurium Infection Patterns along the Murine Intestinal Tract. *Cell Reports*, *27*(9), 2665-2678.e3. https://doi.org/10.1016/j.celrep.2019.04.106

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T., Takahashi, M., Fukuda, N. N., Murakami, S., Miyauchi, E., Hino, S., Atarashi, K., Onawa, S., Fujimura, Y., Lockett, T., … Ohno, H. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*, *504*(7480), 446–450. https://doi.org/10.1038/nature12721

Galan, J. E., & Curtiss, R. (1991). Distribution of the invA, -B, -C, and -D genes of Salmonella typhimurium among other Salmonella serovars: invA mutants of Salmonella typhi are deficient for entry into mammalian cells. *Infection and Immunity*, *59*(9), 2901–2908. https://doi.org/10.1128/iai.59.9.2901-2908.1991

Gallo, R. L., & Hooper, L. V. (2012). Epithelial antimicrobial defence of the skin and intestine. In *Nature Reviews Immunology* (Vol. 12, Issue 7, pp. 503–516). https://doi.org/10.1038/nri3228

Garcia, M. A., Nelson, W. J., & Chavez, N. (2018). Cell–cell junctions organize structural and signaling networks. *Cold Spring Harbor Perspectives in Biology*, *10*(4). https://doi.org/10.1101/cshperspect.a029181

Garg, A., Zhao, A., Erickson, S. L., Mukherjee, S., Lau, A. J., Alston, L., Chang, T. K. H., Mani, S., & Hirota, S. A. (2016). Pregnane x receptor activation attenuates inflammation-associated intestinal epithelial barrier dysfunction by inhibiting cytokine-induced myosin light-chain kinase expression and c-jun n-terminal kinase 1/2 activation. *Journal of Pharmacology and Experimental Therapeutics*, *359*(1), 91–101. https://doi.org/10.1124/jpet.116.234096

Garner, C. D., Antonopoulos, D. A., Wagner, B., Duhamel, G. E., Keresztes, I., Ross, D. A., Young, V. B., & Altier, C. (2009). Perturbation of the small intestine microbial ecology by streptomycin alters pathology in a Salmonella enterica serovar typhimurium murine model of infection. *Infection and Immunity*, *77*(7), 2691–2702. https://doi.org/10.1128/IAI.01570-08

Garrison, W. D., Battle, M. A., Yang, C., Kaestner, K. H., Sladek, F. M., & Duncan, S. A. (2006). Hepatocyte Nuclear Factor 4α Is Essential for Embryonic Development of the Mouse Colon. *Gastroenterology*, *130*(4), 19.e1-19.e. https://doi.org/10.1053/j.gastro.2006.01.003

Gassler, N. (2017). Paneth cells in intestinal physiology and pathophysiology. *World Journal of Gastrointestinal Pathophysiology*, *8*(4), 150–160. https://doi.org/10.4291/wjgp.v8.i4.150

Genta, R. M., Gurer, I. E., Graham, D. Y., Krishnan, B., Segura, A. M., Gutierrez, O., Kim, J. G., & Burchette, J. (1996). Adherence of Helicobacter pylori to areas of incomplete

intestinal metaplasia in the gastric mucosa. *Gastroenterology*, *111*(5), 1206–1211. https://doi.org/10.1053/gast.1996.v111.pm8898634

Ghaleb, A. M., Aggarwal, G., Bialkowska, A. B., Nandan, M. O., & Yang, V. W. (2008). Notch inhibits expression of the Krüppel-like factor 4 tumor suppressor in the intestinal epithelium. *Molecular Cancer Research*, *6*(12), 1920–1927. https://doi.org/10.1158/1541-7786.MCR-08-0224

Gilbert, J. A., Quinn, R. A., Debelius, J., Xu, Z. Z., Morton, J., Garg, N., Jansson, J. K., Dorrestein, P. C., & Knight, R. (2016). Microbiome-wide association studies link dynamic microbial consortia to disease. In *Nature* (Vol. 535, Issue 7610, pp. 94–103). Nature Publishing Group. https://doi.org/10.1038/nature18850

Gill, P. A., van Zelm, M. C., Muir, J. G., & Gibson, P. R. (2018). Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. In *Alimentary Pharmacology and Therapeutics* (Vol. 48, Issue 1, pp. 15–34). Blackwell Publishing Ltd. https://doi.org/10.1111/apt.14689

Glas, J., Wagner, J., Seiderer, J., Olszak, T., Wetzke, M., Beigel, F., Tillack, C., Stallhofer, J., Friedrich, M., Steib, C., Göke, B., Ochsenkühn, T., Karbalai, N., Diegelmann, J., Czamara, D., & Brand, S. (2012). PTPN2 gene variants are associated with susceptibility to both Crohn's disease and ulcerative colitis supporting a common genetic disease background. *PLoS ONE*, *7*(3). https://doi.org/10.1371/journal.pone.0033682

Goswami, P., Das, P., Verma, A. K., Prakash, S., Das, T. K., Nag, T. C., Ahuja, V., Gupta, S. D., & Makharia, G. K. (2014). Are alterations of tight junctions at molecular and ultrastructural level different in duodenal biopsies of patients with celiac disease and Crohn's disease? *Virchows Archiv*, *465*(5), 521–530. https://doi.org/10.1007/s00428-014-1651-1

Goto, Y., Obata, T., Kunisawa, J., Sato, S., Ivanov, I. I., Lamichhane, A., Takeyama, N., Kamioka, M., Sakamoto, M., Matsuki, T., Setoyama, H., Imaoka, A., Uematsu, S., Akira, S., Domino, S. E., Kulig, P., Becher, B., Renauld, J. C., Sasakawa, C., … Kiyono, H. (2014). Innate lymphoid cells regulate intestinal epithelial cell glycosylation. *Science*, *345*(6202). https://doi.org/10.1126/science.1254009

Gowrikumar, S., Ahmad, R., Uppada, S. B., Washington, M. K., Shi, C., Singh, A. B., & Dhawan, P. (2019). Upregulated claudin-1 expression promotes colitis-associated cancer by promoting β-catenin phosphorylation and activation in Notch/p-AKT-dependent manner. *Oncogene*, *38*(26), 5321–5337. https://doi.org/10.1038/s41388-019-0795-5

Graham, D. B., & Xavier, R. J. (2020). Pathway paradigms revealed from the genetics of inflammatory bowel disease. In *Nature* (Vol. 578, Issue 7796, pp. 527–539). Nature Research. https://doi.org/10.1038/s41586-020-2025-2

Gregorieff, A., & Clevers, H. (2005). Wnt signaling in the intestinal epithelium: From endoderm to cancer. In *Genes and Development* (Vol. 19, Issue 8, pp. 877–890). https://doi.org/10.1101/gad.1295405

Gregorieff, A., Stange, D. E., Kujala, P., Begthel, H., van den Born, M., Korving, J., Peters, P. J., & Clevers, H. (2009). The Ets-Domain Transcription Factor Spdef Promotes Maturation of Goblet and Paneth Cells in the Intestinal Epithelium. *Gastroenterology*, *137*(4). https://doi.org/10.1053/j.gastro.2009.06.044

Groschwitz, K. R., & Hogan, S. P. (2009). Intestinal barrier function: Molecular regulation and disease pathogenesis. In *Journal of Allergy and Clinical Immunology* (Vol. 124,

Issue 1, pp. 3–20). https://doi.org/10.1016/j.jaci.2009.05.038

Guan, Q. (2019). A Comprehensive Review and Update on the Pathogenesis of Inflammatory Bowel Disease. *Journal of Immunology Research*, *2019*. https://doi.org/10.1155/2019/7247238

Günzel, D., & Yu, A. S. L. (2013). Claudins and the modulation of tight junction permeability. *Physiological Reviews*, *93*(2), 525–569. https://doi.org/10.1152/physrev.00019.2012

Gurney, M. A., Laubitz, D., Ghishan, F. K., & Kiela, P. R. (2017). Pathophysiology of Intestinal Na+/H+ Exchange. In *CMGH* (Vol. 3, Issue 1, pp. 27–40). Elsevier Inc. https://doi.org/10.1016/j.jcmgh.2016.09.010

Gustafsson, J. K., Ermund, A., Ambort, D., Johansson, M. E. V., Nilsson, H. E., Thorell, K., Hebert, H., Sjövall, H., & Hansson, G. C. (2012). Bicarbonate and functional CFTR channel are required for proper mucin secretion and link cystic fibrosis with its mucus phenotype. *Journal of Experimental Medicine*, *209*(7), 1263–1272. https://doi.org/10.1084/jem.20120562

Hall, A. B., Tolonen, A. C., & Xavier, R. J. (2017). Human genetic variation and the gut microbiome in disease. In *Nature Reviews Genetics* (Vol. 18, Issue 11, pp. 690–699). Nature Publishing Group. https://doi.org/10.1038/nrg.2017.63

Halpern, M. D., & Denning, P. W. (2015). The role of intestinal epithelial barrier function in the development of NEC. *Tissue Barriers*. https://doi.org/10.1080/21688370.2014.1000707

Hamid, N., & Jain, S. K. (2008). Characterization of an outer membrane protein of Salmonella enterica serovar Typhimurium that confers protection against typhoid. *Clinical and Vaccine Immunology*, *15*(9), 1461–1471. https://doi.org/10.1128/CVI.00093-08

Hayhurst, G. P., Lee, Y.-H., Lambert, G., Ward, J. M., & Gonzalez, F. J. (2001). Hepatocyte Nuclear Factor 4α (Nuclear Receptor 2A1) Is Essential for Maintenance of Hepatic Gene Expression and Lipid Homeostasis. *Molecular and Cellular Biology*, *21*(4), 1393–1403. https://doi.org/10.1128/mcb.21.4.1393-1403.2001

He, W., Wang, M. L., Jiang, H. Q., Steppan, C. M., Shin, M. E., Thurnheer, M. C., Cebra, J. J., Lazar, M. A., & Wu, G. D. (2003). Bacterial Colonization Leads to the Colonic Secretion of RELMβ/FIZZ2, a Novel Goblet Cell-Specific Protein. *Gastroenterology*, *125*(5), 1388–1397. https://doi.org/10.1016/j.gastro.2003.07.009

He, X. C., Zhang, J., Tong, W. G., Tawfik, O., Ross, J., Scoville, D. H., Tian, Q., Zeng, X., He, X., Wiedemann, L. M., Mishina, Y., & Li, L. (2004). BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-β-catenin signaling. *Nature Genetics*, *36*(10), 1117–1121. https://doi.org/10.1038/ng1430

Heazlewood, C. K., Cook, M. C., Eri, R., Price, G. R., Tauro, S. B., Taupin, D., Thornton, D. J., Chin, W. P., Crockford, T. L., Cornall, R. J., Adams, R., Kato, M., Nelms, K. A., Hong, N. A., Florin, T. H. J., Goodnow, C. C., & McGuckin, M. A. (2008). Aberrant mucin assembly in mice causes endoplasmic reticulum stress and spontaneous inflammation resembling ulcerative colitis. *PLoS Medicine*, *5*(3), 0440–0460. https://doi.org/10.1371/journal.pmed.0050054

Hedin, C. R., McCarthy, N. E., Louis, P., Farquharson, F. M., McCartney, S., Taylor, K., Prescott, N. J., Murrells, T., Stagg, A. J., Whelan, K., & Lindsay, J. O. (2014). Altered intestinal microbiota and blood T cell phenotype are shared by patients with Crohn's disease and their unaffected siblings. *Gut*, *63*(10), 1578–1586.

https://doi.org/10.1136/gutjnl-2013-306226

Hedin, C., Van Der Gast, C. J., Rogers, G. B., Cuthbertson, L., McCartney, S., Stagg, A. J., Lindsay, J. O., & Whelan, K. (2016). Siblings of patients with Crohn's disease exhibit a biologically relevant dysbiosis in mucosal microbial metacommunities. *Gut*, *65*(6), 944–953. https://doi.org/10.1136/gutjnl-2014-308896

Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, M. L., Forssberg, H., & Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(7), 3047–3052. https://doi.org/10.1073/pnas.1010529108

Heller, F., Florian, P., Bojarski, C., Richter, J., Christ, M., Hillenbrand, B., Mankertz, J., Gitter, A. H., Bürgel, N., Fromm, M., Zeitz, M., Fuss, I., Strober, W., & Schulzke, J. D. (2005). Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology*, *129*(2), 550–564. https://doi.org/10.1016/j.gastro.2005.05.002

Henke, M. T., Kenny, D. J., Cassilly, C. D., Vlamakis, H., Xavier, R. J., & Clardy, J. (2019). Ruminococcus gnavus, a member of the human gut microbiome associated with Crohn's disease, produces an inflammatory polysaccharide. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(26), 12672–12677. https://doi.org/10.1073/pnas.1904099116

Herbert, D. R., Yang, J. Q., Hogan, S. P., Groschwitz, K., Khodoun, M., Munitz, A., Orekov, T., Perkins, C., Wang, Q., Brombacher, F., Urban, J. F., Rothenberg, M. E., & Finkelman, F. D. (2009). Intestinal epithelial cell secretion of RELM-β protects against gastrointestinal worm infection. *Journal of Experimental Medicine*, *206*(13), 2947–2957. https://doi.org/10.1084/jem.20091268

Hering, N. A., Fromm, M., & Schulzke, J. D. (2012). Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. In *Journal of Physiology* (Vol. 590, Issue 5, pp. 1035–1044). https://doi.org/10.1113/jphysiol.2011.224568

Hollander, D. (1988). Crohn's disease - A permeability disorder of the tight junction. In *Gut* (Vol. 29, Issue 12, pp. 1621–1624). BMJ Publishing Group. https://doi.org/10.1136/gut.29.12.1621

Holmes, J. L., Van Itallie, C. M., Rasmussen, J. E., & Anderson, J. M. (2006). Claudin profiling in the mouse during postnatal intestinal development and along the gastrointestinal tract reveals complex expression patterns. *Gene Expression Patterns*, *6*(6), 581–588. https://doi.org/10.1016/j.modgep.2005.12.001

Hu, Y. J., Wang, Y. D., Tan, F. Q., & Yang, W. X. (2013). Regulation of paracellular permeability: Factors and mechanisms. *Molecular Biology Reports*, *40*(11), 6123–6142. https://doi.org/10.1007/s11033-013-2724-y

Ijssennagger, N., van der Meer, R., & van Mil, S. W. C. (2016). Sulfide as a Mucus Barrier-Breaker in Inflammatory Bowel Disease? In *Trends in Molecular Medicine* (Vol. 22, Issue 3, pp. 190–199). Elsevier Ltd. https://doi.org/10.1016/j.molmed.2016.01.002

Imhann, F., Vich Vila, A., Bonder, M. J., Fu, J., Gevers, Di., Visschedijk, M. C., Spekhorst, L. M., Alberts, R., Franke, L., Van Dullemen, H. M., Ter Steege, R. W. F., Huttenhower, C., DIjkstra, G., Xavier, R. J., Festen, E. A. M., Wijmenga, C., Zhernakova, A., & Weersma, R. K. (2018). Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. *Gut*, *67*(1), 108–119. https://doi.org/10.1136/gutjnl-2016-312135

In, J. G., Foulke-Abel, J., Clarke, E., & Kovbasnjuk, O. (2019). Human colonoid monolayers to study interactions between pathogens, commensals, and host intestinal epithelium. *Journal of Visualized Experiments*, *2019*(146). https://doi.org/10.3791/59357

Iorga, A., Cunningham, C. M., Moazeni, S., Ruffenach, G., Umar, S., & Eghbali, M. (2017). The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. In *Biology of sex differences* (Vol. 8, Issue 1, p. 33). https://doi.org/10.1186/s13293-017-0152-8

Ivanov, A. I., & Naydenov, N. G. (2013). Dynamics and Regulation of Epithelial Adherens Junctions. Recent Discoveries and Controversies. In *International Review of Cell and Molecular Biology* (Vol. 303, pp. 27–99). Elsevier Inc. https://doi.org/10.1016/B978-0-12-407697-6.00002-7

Jakobsson, H. E., Rodríguez-Piñeiro, A. M., Schütte, A., Ermund, A., Boysen, P., Bemark, M., Sommer, F., Bäckhed, F., Hansson, G. C., & Johansson, M. E. (2015). The composition of the gut microbiota shapes the colon mucus barrier. *EMBO Reports*, *16*(2), 164–177. https://doi.org/10.15252/embr.201439263

Jiang, S., Tanaka, T., Iwanari, H., Hotta, H., Yamashita, H., Kumakura, J., Wanatabe, Y., Uchiyama, Y., Aburatani, H., Hamakubo, T., Kodama, T., & Naito, M. (2003). Expression and localization of P1 promoter-driven hepatocyte nuclear factor-4α (HNF4α) isoforms in human and rats. *Nuclear Receptor*, *1*. https://doi.org/10.1186/1478-1336-1-5

JN, R., JY., W., & Rafael, S. (2010). Intestinal Architecture and Development. *Regulation of Gastrointestinal Mucosal Growth.*, 1–5. http://www.ncbi.nlm.nih.gov/books/NBK54098/#s2_2

Johansson, M. E. V. (2012). Fast renewal of the distal colonic mucus layers by the surface goblet cells as measured by in vivo labeling of mucin glycoproteins. *PLoS ONE*, *7*(7). https://doi.org/10.1371/journal.pone.0041009

Johansson, M. E. V., Gustafsson, J. K., Holmen-Larsson, J., Jabbar, K. S., Xia, L., Xu, H., Ghishan, F. K., Carvalho, F. A., Gewirtz, A. T., Sjovall, H., & Hansson, G. C. (2014). Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. *Gut*, *63*(2), 281–291. https://doi.org/10.1136/gutjnl-2012-303207

Johansson, M. E. V., & Hansson, G. C. (2016). Immunological aspects of intestinal mucus and mucins. In *Nature Reviews Immunology* (Vol. 16, Issue 10, pp. 639–649). Nature Publishing Group. https://doi.org/10.1038/nri.2016.88

Johansson, M. E. V., Phillipson, M., Petersson, J., Velcich, A., Holm, L., & Hansson, G. C. (2008). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(39), 15064–15069. https://doi.org/10.1073/pnas.0803124105

Jostins, L., Ripke, S., Weersma, R. K., Duerr, R. H., McGovern, D. P., Hui, K. Y., Lee, J. C., Philip Schumm, L., Sharma, Y., Anderson, C. A., Essers, J., Mitrovic, M., Ning, K., Cleynen, I., Theatre, E., Spain, S. L., Raychaudhuri, S., Goyette, P., Wei, Z., … Whittaker, P. (2012). Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*, *491*(7422), 119–124. https://doi.org/10.1038/nature11582

Kadesch, T. (2004). Notch signaling: The demise of elegant simplicity. In *Current Opinion in Genetics and Development* (Vol. 14, Issue 5, pp. 506–512). https://doi.org/10.1016/j.gde.2004.07.007

Kalischuk, L. D., & Buret, A. G. (2010). A role for Campylobacter jejuni-induced enteritis in inflammatory bowel disease? In *American Journal of Physiology - Gastrointestinal and Liver Physiology* (Vol. 298, Issue 1). https://doi.org/10.1152/ajpgi.00193.2009

Kamekura, R., Nava, P., Feng, M., Quiros, M., Nishio, H., Weber, D. A., Parkos, C. A., & Nusrat, A. (2015). Inflammation-induced desmoglein-2 ectodomain shedding compromises the mucosal barrier. *Molecular Biology of the Cell*, *26*(18), 3165–3177. https://doi.org/10.1091/mbc.E15-03-0147

Kaplan, G. G., & Ng, S. C. (2017). Understanding and Preventing the Global Increase of Inflammatory Bowel Disease. *Gastroenterology*, *152*(2), 313-321.e2. https://doi.org/10.1053/j.gastro.2016.10.020

Karam, S. M. (1999). Lineage commitment and maturation of epithelial cells in the gut. *Frontiers in Bioscience*, *4*(1–3), d286. https://doi.org/10.2741/karam

Kato, K., Lillehoj, E. P., Lu, W., & Kim, K. C. (2017). MUC1: The First Respiratory Mucin with an Anti-Inflammatory Function. *Journal of Clinical Medicine*, *6*(12), 110. https://doi.org/10.3390/jcm6120110

Katoh, M., & Katoh, M. (2007). WNT signaling pathway and stem cell signaling network. In *Clinical Cancer Research* (Vol. 13, Issue 14, pp. 4042–4045). https://doi.org/10.1158/1078-0432.CCR-06-2316

Khan, I., Ullah, N., Zha, L., Bai, Y., Khan, A., Zhao, T., Che, T., & Zhang, C. (2019). Alteration of gut microbiota in inflammatory bowel disease (IBD): Cause or consequence? IBD treatment targeting the gut microbiome. In *Pathogens* (Vol. 8, Issue 3). MDPI AG. https://doi.org/10.3390/pathogens8030126

Khor, B., Gardet, A., & Xavier, R. J. (2011). Genetics and pathogenesis of inflammatory bowel disease. In *Nature* (Vol. 474, Issue 7351, pp. 307–317). https://doi.org/10.1038/nature10209

Kim, Yong Sung, Unno, T., Kim, B. Y., & Park, M. S. (2020). Sex differences in gut microbiota. In *World Journal of Men's Health* (Vol. 38, Issue 1, pp. 48–60). Korean Society for Sexual Medicine and Andrology. https://doi.org/10.5534/wjmh.190009

Kim, Young S., & Ho, S. B. (2010). Intestinal goblet cells and mucins in health and disease: Recent insights and progress. In *Current Gastroenterology Reports* (Vol. 12, Issue 5, pp. 319–330). https://doi.org/10.1007/s11894-010-0131-2

Kinugasa, T., Sakaguchi, T., Gu, X., & Reinecker, H. C. (2000). Claudins regulate the intestinal barrier in response to immune mediators. *Gastroenterology*, *118*(6), 1001–1011. https://doi.org/10.1016/S0016-5085(00)70351-9

Kjellev, S. (2009). The trefoil factor family - Small peptides with multiple functionalities. In *Cellular and Molecular Life Sciences* (Vol. 66, Issue 8, pp. 1350–1369). https://doi.org/10.1007/s00018-008-8646-5

Klepsch, V., Gerner, R. R., Klepsch, S., Olson, W. J., Tilg, H., Moschen, A. R., Baier, G., & Hermann-Kleiter, N. (2018). Nuclear orphan receptor NR2F6 as a safeguard against experimental murine colitis. *Gut*, *67*(8), 1434–1444. https://doi.org/10.1136/gutjnl-2016-313466

Klepsch, V., Moschen, A. R., Tilg, H., Baier, G., & Hermann-Kleiter, N. (2019). Nuclear receptors regulate intestinal inflammation in the context of IBD. In *Frontiers in Immunology* (Vol. 10, Issue MAY). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2019.01070

Knoop, K. A., & Newberry, R. D. (2018). Goblet cells: multifaceted players in immunity at mucosal surfaces. In *Mucosal Immunology* (Vol. 11, Issue 6, pp. 1551–1557). Nature

Publishing Group. https://doi.org/10.1038/s41385-018-0039-y

Kong, S., Zhang, Y. H., & Zhang, W. (2018). Regulation of intestinal epithelial cells properties and functions by amino acids. In *BioMed Research International* (Vol. 2018). Hindawi Limited. https://doi.org/10.1155/2018/2819154

Kopan, R., & Ilagan, M. X. G. (2009). The Canonical Notch Signaling Pathway: Unfolding the Activation Mechanism. In *Cell* (Vol. 137, Issue 2, pp. 216–233). https://doi.org/10.1016/j.cell.2009.03.045

Kostic, A. D., Xavier, R. J., & Gevers, D. (2014). The microbiome in inflammatory bowel disease: Current status and the future ahead. *Gastroenterology*, *146*(6), 1489–1499. https://doi.org/10.1053/j.gastro.2014.02.009

Kyo, K., Muto, T., Nagawa, H., Lathrop, G. M., & Nakamura, Y. (2001). Associations of distinct variants of the intestinal mucin gene MUC3A with ulcerative colitis and Crohn's disease. *Journal of Human Genetics*, *46*(1), 5–20. https://doi.org/10.1007/s100380170118

Lameris, A. L., Huybers, S., Kaukinen, K., Mäkelä, T. H., Bindels, R. J., Hoenderop, J. G., & Nevalainen, P. I. (2013). Expression profiling of claudins in the human gastrointestinal tract in health and during inflammatory bowel disease. *Scandinavian Journal of Gastroenterology*, *48*(1), 58–69. https://doi.org/10.3109/00365521.2012.741616

Lang, T., Hansson, G. C., & Samuelsson, T. (2007). Gel-forming mucins appeared early in metazoan evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(41), 16209–16214. https://doi.org/10.1073/pnas.0705984104

Larsson, J. M. H., Karlsson, H., Crespo, J. G., Johansson, M. E. V., Eklund, L., Sjövall, H., & Hansson, G. C. (2011). Altered O-glycosylation profile of MUC2 mucin occurs in active ulcerative colitis and is associated with increased inflammation. *Inflammatory Bowel Diseases*, *17*(11), 2299–2307. https://doi.org/10.1002/ibd.21625

Lee, B., Moon, K. M., & Kim, C. Y. (2018). Tight junction in the intestinal epithelium: Its association with diseases and regulation by phytochemicals. In *Journal of Immunology Research* (Vol. 2018). Hindawi Limited. https://doi.org/10.1155/2018/2645465

Lee, J. G., Han, D. S., Jo, S. V., Reum Lee, A., Park, C. H., Eun, C. S., & Lee, Y. (2019). Characteristics and pathogenic role of adherent-invasive Escherichia coli in inflammatory bowel disease: Potential impact on clinical outcomes. *PLoS ONE*, *14*(4). https://doi.org/10.1371/journal.pone.0216165

Lee, S. H. (2015). Intestinal Permeability Regulation by Tight Junction: Implication on Inflammatory Bowel Diseases. *Intestinal Research*, *13*(1), 11. https://doi.org/10.5217/ir.2015.13.1.11

Lencer, W. I., Cheung, G., Strohmeier, G. R., Currie, M. G., Ouellette, A. J., Selsted, M. E., & Madara, J. L. (1997). Induction of epithelial chloride secretion by channel-forming cryptdins 2 and 3. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(16), 8585–8589. https://doi.org/10.1073/pnas.94.16.8585

Levy, M., Kolodziejczyk, A. A., Thaiss, C. A., & Elinav, E. (2017). Dysbiosis and the immune system. In *Nature Reviews Immunology* (Vol. 17, Issue 4, pp. 219–232). Nature Publishing Group. https://doi.org/10.1038/nri.2017.7

Lhocine, N., Arena, E. T., Bomme, P., Ubelmann, F., Prévost, M. C., Robine, S., & Sansonetti, P. J. (2015). Apical invasion of intestinal epithelial cells by salmonella typhimurium requires villin to remodel the brush border actin cytoskeleton. *Cell Host and Microbe*, *17*(2), 164–177. https://doi.org/10.1016/j.chom.2014.12.003

Li, B., Lee, C., Cadete, M., Zhu, H., Koike, Y., Hock, A., Wu, R. Y., Botts, S. R., Minich, A., Alganabi, M., Chi, L., Zani-Ruttenstock, E., Miyake, H., Chen, Y., Mutanen, A., Ngan, B., Johnson-Henry, K. C., De Coppi, P., Eaton, S., … Pierro, A. (2019). Impaired Wnt/β-catenin pathway leads to dysfunction of intestinal regeneration during necrotizing enterocolitis. *Cell Death and Disease*, *10*(10). https://doi.org/10.1038/s41419-019-1987-1

Li, Q., Wang, C., Tang, C., He, Q., Li, N., & Li, J. (2014). Dysbiosis of gut fungal microbiota is associated with mucosal inflammation in crohn's disease. *Journal of Clinical Gastroenterology*, *48*(6), 513–523. https://doi.org/10.1097/MCG.0000000000000035

Lili, L. N., Farkas, A. E., Gerner-Smidt, C., Overgaard, C. E., Moreno, C. S., Parkos, C. A., Capaldo, C. T., & Nusrat, A. (2016). Claudin-based barrier differentiation in the colonic epithelial crypt niche involves Hopx/Klf4 and Tcf7l2/Hnf4-α cascades. *Tissue Barriers*, *4*(3). https://doi.org/10.1080/21688370.2016.1214038

Lindén, S. K., Florin, T. H. J., & McGuckin, M. A. (2008). Mucin dynamics in intestinal bacterial infection. *PLoS ONE*, *3*(12). https://doi.org/10.1371/journal.pone.0003952

Liu, J. Z., Van Sommeren, S., Huang, H., Ng, S. C., Alberts, R., Takahashi, A., Ripke, S., Lee, J. C., Jostins, L., Shah, T., Abedian, S., Cheon, J. H., Cho, J., Daryani, N. E., Franke, L., Fuyuno, Y., Hart, A., Juyal, R. C., Juyal, G., … Weersma, R. K. (2015). Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nature Genetics*, *47*(9), 979–986. https://doi.org/10.1038/ng.3359

Liu, S., Zhao, W., Lan, P., & Mou, X. (2020). The microbiome in inflammatory bowel diseases: from pathogenesis to therapy. In *Protein and Cell*. Higher Education Press. https://doi.org/10.1007/s13238-020-00745-3

Lloyd-Price, J., Arze, C., Ananthakrishnan, A. N., Schirmer, M., Avila-Pacheco, J., Poon, T. W., Andrews, E., Ajami, N. J., Bonham, K. S., Brislawn, C. J., Casero, D., Courtney, H., Gonzalez, A., Graeber, T. G., Hall, A. B., Lake, K., Landers, C. J., Mallick, H., Plichta, D. R., … Huttenhower, C. (2019). Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature*, *569*(7758), 655–662. https://doi.org/10.1038/s41586-019-1237-9

Longman, R. J., Poulsom, R., Corfield, A. P., Warren, B. F., Wright, N. A., & Thomas, M. G. (2006). Alterations in the composition of the supramucosal defense barrier in relation to disease severity of ulcerative colitis. *Journal of Histochemistry and Cytochemistry*, *54*(12), 1335–1348. https://doi.org/10.1369/jhc.5A6904.2006

Loubinoux, J., Bronowicki, J. P., Pereira, I. A. C., Mougenel, J. L., & Le Faou, A. E. (2002). Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. *FEMS Microbiology Ecology*, *40*(2), 107–112. https://doi.org/10.1016/S0168-6496(02)00201-5

Luettig, J., Rosenthal, R., Barmeyer, C., & Schulzke, J. D. (2015). Claudin-2 as a mediator of leaky gut barrier during intestinal inflammation. In *Tissue Barriers* (Vol. 3, Issue 1). Taylor and Francis Inc. https://doi.org/10.4161/21688370.2014.977176

Luissint, A. C., Nusrat, A., & Parkos, C. A. (2014). JAM-related proteins in mucosal homeostasis and inflammation. In *Seminars in Immunopathology* (Vol. 36, Issue 2, pp. 211–226). Springer Verlag. https://doi.org/10.1007/s00281-014-0421-0

Luissint, A. C., Parkos, C. A., & Nusrat, A. (2016). Inflammation and the Intestinal Barrier: Leukocyte–Epithelial Cell Interactions, Cell Junction Remodeling, and Mucosal Repair. In *Gastroenterology* (Vol. 151, Issue 4, pp. 616–632). W.B. Saunders.

https://doi.org/10.1053/j.gastro.2016.07.008

Lussier, C. R., Babeu, J. P., Auclair, B. A., Perreault, N., & Boudreau, F. (2008). Hepatocyte nuclear factor-4α promotes differentiation of intestinal epithelial cells in a coculture system. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *294*(2). https://doi.org/10.1152/ajpgi.00418.2007

Mack, D. R., Ahrne, S., Hyde, L., Wei, S., & Hollingsworth, M. A. (2003). Extracellular MUC3 mucin secretion follows adherence of Lactobacillus strains to intestinal epithelial cells in vitro. *Gut*, *52*(6), 827–833. https://doi.org/10.1136/gut.52.6.827

Mack, David R., Michail, S., Wei, S., McDougall, L., & Hollingsworth, M. A. (1999). Probiotics inhibit enteropathogenic E. coli adherence in vitro by inducing intestinal mucin gene expression. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *276*(4 39-4). https://doi.org/10.1152/ajpgi.1999.276.4.g941

Magnúsdóttir, S., Ravcheev, D., De Crécy-Lagard, V., & Thiele, I. (2015). Systematic genome assessment of B-vitamin biosynthesis suggests cooperation among gut microbes. *Frontiers in Genetics*, *6*(MAR). https://doi.org/10.3389/fgene.2015.00148

Mahendran, V., Liu, F., Riordan, S. M., Grimm, M. C., Tanaka, M. M., & Zhang, L. (2016). Examination of the effects of Campylobacter concisus zonula occludens toxin on intestinal epithelial cells and macrophages. *Gut Pathogens*, *8*(1). https://doi.org/10.1186/s13099-016-0101-9

Mahendran, V., Riordan, S. M., Grimm, M. C., Tran, T. A. T., Major, J., Kaakoush, N. O., Mitchell, H., & Zhang, L. (2011). Prevalence of Campylobacter species in adult Crohn's disease and the preferential colonization sites of Campylobacter species in the human intestine. *PLoS ONE*, *6*(9). https://doi.org/10.1371/journal.pone.0025417

Mankertz, J., Amasheh, M., Krug, S. M., Fromm, A., Amasheh, S., Hillenbrand, B., Tavalali, S., Fromm, M., & Schulzke, J. D. (2009). TNFα up-regulates claudin-2 expression in epithelial HT-29/B6 cells via phosphatidylinositol-3-kinase signaling. *Cell and Tissue Research*, *336*(1), 67–77. https://doi.org/10.1007/s00441-009-0751-8

Mankertz, Joachim, & Schulzke, J. D. (2007). Altered permeability in inflammatory bowel disease: Pathophysiology and clinical implications. In *Current Opinion in Gastroenterology* (Vol. 23, Issue 4, pp. 379–383). https://doi.org/10.1097/MOG.0b013e32816aa392

Mar, J. S., Lamere, B. J., Lin, D. L., Levan, S., Nazareth, M., Mahadevan, U., & Lynch, S. V. (2016). Disease severity and immune activity relate to distinct interkingdom gut microbiome states in ethnically distinct ulcerative colitis patients. *MBio*, *7*(4). https://doi.org/10.1128/mBio.01072-16

Marchiando, A. M., Shen, L., Vallen Graham, W., Weber, C. R., Schwarz, B. T., Austin, J. R., Raleigh, D. R., Guan, Y., Watson, A. J. M., Montrose, M. H., & Turner, J. R. (2010). Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation in vivo. *Journal of Cell Biology*, *189*(1), 111–126. https://doi.org/10.1083/jcb.200902153

Marcil, V., Sinnett, D., Seidman, E., Boudreau, F., Gendron, F. P., Beaulieu, J. F., Menard, D., Lambert, M., Bitton, A., Sanchez, R., Amre, D., & Levy, E. (2012). Association between genetic variants in the HNF4A gene and childhood-onset Crohns disease. *Genes and Immunity*, *13*(7), 556–565. https://doi.org/10.1038/gene.2012.37

Martini, E., Krug, S. M., Siegmund, B., Neurath, M. F., & Becker, C. (2017). Mend Your Fences: The Epithelial Barrier and its Relationship With Mucosal Immunity in Inflammatory Bowel Disease. In *CMGH* (Vol. 4, Issue 1, pp. 33–46). Elsevier Inc.

https://doi.org/10.1016/j.jcmgh.2017.03.007
Martinoli, C., Chiavelli, A., & Rescigno, M. (2007). Entry Route of Salmonella typhimurium Directs the Type of Induced Immune Response. *Immunity*, *27*(6), 975–984. https://doi.org/10.1016/j.immuni.2007.10.011
Mathias, A., Pais, B., Favre, L., Benyacoub, J., & Corthésy, B. (2015). Role of secretory IgA in the mucosal sensing of commensal bacteria. In *Gut Microbes* (Vol. 5, Issue 6, pp. 688–695). Landes Bioscience. https://doi.org/10.4161/19490976.2014.983763
Matsuo, K., Ota, H., Akamatsu, T., Sugiyama, A., & Katsuyama, T. (1997). Histochemistry of the surface mucous gel layer of the human colon. *Gut*, *40*(6), 782–789. https://doi.org/10.1136/gut.40.6.782
Maynard, C. L., Elson, C. O., Hatton, R. D., & Weaver, C. T. (2012). Reciprocal interactions of the intestinal microbiota and immune system. In *Nature* (Vol. 489, Issue 7415, pp. 231–241). https://doi.org/10.1038/nature11551
McCole, D. F. (2014). IBD candidate genes and intestinal barrier regulation. In *Inflammatory Bowel Diseases* (Vol. 20, Issue 10, pp. 1829–1849). Lippincott Williams and Wilkins. https://doi.org/10.1097/MIB.0000000000000090
McGovern, D. P. B., Gardet, A., Törkvist, L., Goyette, P., Essers, J., Taylor, K. D., Neale, B. M., Ong, R. T. H., Lagacé, C., Li, C., Green, T., Stevens, C. R., Beauchamp, C., Fleshner, P. R., Carlson, M., D'Amato, M., Halfvarson, J., Hibberd, M. L., Lördal, M., … Seielstad, M. (2010). Genome-wide association identifies multiple ulcerative colitis susceptibility loci. *Nature Genetics*, *42*(4), 332–337. https://doi.org/10.1038/ng.549
McGuckin, M. A., Eri, R., Simms, L. A., Florin, T. H. J., & Radford-Smith, G. (2009). Intestinal barrier dysfunction in inflammatory bowel diseases. *Inflammatory Bowel Diseases*. https://doi.org/10.1002/ibd.20539
McGuckin, M. A., Lindén, S. K., Sutton, P., & Florin, T. H. (2011). Mucin dynamics and enteric pathogens. *Nature Reviews Microbiology*, *9*(4), 265–278. https://doi.org/10.1038/nrmicro2538
McLoughlin, K., Schluter, J., Rakoff-Nahoum, S., Smith, A. L., & Foster, K. R. (2016). Host Selection of Microbiota via Differential Adhesion. *Cell Host and Microbe*, *19*(4), 550–559. https://doi.org/10.1016/j.chom.2016.02.021
Meyer, T. N., Schwesinger, C., & Denker, B. M. (2002). Zonula occludens-1 is a scaffolding protein for signaling molecules: Gα12 directly binds to the Src homology 3 domain and regulates paracellular permeability in epithelial cells. *Journal of Biological Chemistry*, *277*(28), 24855–24858. https://doi.org/10.1074/jbc.C200240200
Meyerholz, D. K., Stabel, T. J., Ackermann, M. R., Carlson, S. A., Jones, B. D., & Pohlenz, J. (2002). Early Epithelial Invasion by Salmonella enterica Serovar Typhimurium DT104 in the Swine Ileum. *Veterinary Pathology*, *39*(6), 712–720. https://doi.org/10.1354/vp.39-6-712
Michetti, P., Mahan, M. J., Slauch, J. M., Mekalanos, J. J., & Neutra, M. R. (1992). Monoclonal secretory immunoglobulin A protects mice against oral challenge with the invasive pathogen Salmonella typhimurium. *Infection and Immunity*, *60*(5), 1786–1792. https://doi.org/10.1128/iai.60.5.1786-1792.1992
Michielan, A., & D'Incà, R. (2015). Intestinal Permeability in Inflammatory Bowel Disease: Pathogenesis, Clinical Evaluation, and Therapy of Leaky Gut. In *Mediators of Inflammation* (Vol. 2015). Hindawi Publishing Corporation. https://doi.org/10.1155/2015/628157
Mills, D. J. S., Tuohy, K. M., Booth, J., Buck, M., Crabbe, M. J. C., Gibson, G. R., & Ames,

J. M. (2008). Dietary glycated protein modulates the colonic microbiota towards a more detrimental composition in ulcerative colitis patients and non-ulcerative colitis subjects. *Journal of Applied Microbiology*, *105*(3), 706–714. https://doi.org/10.1111/j.1365-2672.2008.03783.x

Moehle, C., Ackermann, N., Langmann, T., Aslanidis, C., Kel, A., Kel-Margoulis, O., Schmitz-Madry, A., Zahn, A., Stremmel, W., & Schmitz, G. (2006). Aberrant intestinal expression and allelic variants of mucin genes associated with inflammatory bowel disease. *Journal of Molecular Medicine*, *84*(12), 1055–1066. https://doi.org/10.1007/s00109-006-0100-2

Moor, K., Diard, M., Sellin, M. E., Felmy, B., Wotzka, S. Y., Toska, A., Bakkeren, E., Arnoldini, M., Bansept, F., Co, A. D., Völler, T., Minola, A., Fernandez-Rodriguez, B., Agatic, G., Barbieri, S., Piccoli, L., Casiraghi, C., Corti, D., Lanzavecchia, A., … Slack, E. (2017). High-avidity IgA protects the intestine by enchaining growing bacteria. *Nature*, *544*(7651), 498–502. https://doi.org/10.1038/nature22058

Mottram, L., Wiklund, G., Larson, G., Qadri, F., & Svennerholm, A. M. (2017). FUT2 non-secretor status is associated with altered susceptibility to symptomatic enterotoxigenic Escherichia coli infection in Bangladeshis. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-10854-5

Mucida, D., Park, Y., Kim, G., Turovskaya, O., Scott, I., Kronenberg, M., & Cheroutre, H. (2007). Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science*, *317*(5835), 256–260. https://doi.org/10.1126/science.1145697

Muise, A. M., Walters, T. D., Glowacka, W. K., Griffiths, A. M., Ngan, B. Y., Lan, H., Xu, W., Silverberg, M. S., & Rotin, D. (2009). Polymorphisms in E-cadherin (CDH1) result in a mislocalised cytoplasmic protein that is associated with Crohn's disease. *Gut*, *58*(8), 1121–1127. https://doi.org/10.1136/gut.2008.175117

Mukherjee, S., & Hooper, L. V. (2015). Antimicrobial Defense of the Intestine. In *Immunity* (Vol. 42, Issue 1, pp. 28–39). Cell Press. https://doi.org/10.1016/j.immuni.2014.12.028

Mukherjee, S., Zheng, H., Derebe, M. G., Callenberg, K. M., Partch, C. L., Rollins, D., Propheter, D. C., Rizo, J., Grabe, M., Jiang, Q. X., & Hooper, L. V. (2014). Antibacterial membrane attack by a pore-forming intestinal C-type lectin. *Nature*, *505*(7481), 103–107. https://doi.org/10.1038/nature12729

Müller, A. A., Dolowschiak, T., Sellin, M. E., Felmy, B., Verbree, C., Gadient, S., Westermann, A. J., Vogel, J., LeibundGut-Landmann, S., & Hardt, W. D. (2016). An NK Cell Perforin Response Elicited via IL-18 Controls Mucosal Inflammation Kinetics during Salmonella Gut Infection. *PLoS Pathogens*, *12*(6). https://doi.org/10.1371/journal.ppat.1005723

Muthusamy, S., Jeong, J. J., Cheng, M., Bonzo, J. A., Kumar, A., Gonzalez, F. J., Borthakur, A., Dudeja, P. K., Saksena, S., & Malakooti, J. (2018). Hepatocyte nuclear factor 4α regulates the expression of intestinal epithelial Na+/H+ exchanger isoform 3. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *314*(1), G14–G21. https://doi.org/10.1152/ajpgi.00225.2017

Nair, M. G., Guild, K. J., Du, Y., Zaph, C., Yancopoulos, G. D., Valenzuela, D. M., Murphy, A., Stevens, S., Karow, M., & Artis, D. (2008). Goblet Cell-Derived Resistin-Like Molecule β Augments CD4 + T Cell Production of IFN-γ and Infection-Induced Intestinal Inflammation. *The Journal of Immunology*, *181*(7), 4709–4715. https://doi.org/10.4049/jimmunol.181.7.4709

Napolitano, L. M., Koruda, M. J., Meyer, A. A., & Baker, C. C. (1996). The impact of femur

fracture with associated soft tissue injury on immune function and intestinal permeability. *Shock*, *5*(3), 202–207. https://doi.org/10.1097/00024382-199603000-00006

Nelson, W. J., & Nusse, R. (2004). Convergence of Wnt, β-Catenin, and Cadherin pathways. In *Science* (Vol. 303, Issue 5663, pp. 1483–1487). https://doi.org/10.1126/science.1094291

Nguyen, G. C. (2011). Editorial: Bugs and drugs: Insights into the pathogenesis of inflammatory bowel disease. In *American Journal of Gastroenterology* (Vol. 106, Issue 12, pp. 2143–2145). https://doi.org/10.1038/ajg.2011.308

Nie, X., Xie, R., & Tuo, B. (2018). Effects of Estrogen on the Gastrointestinal Tract. In *Digestive Diseases and Sciences* (Vol. 63, Issue 3, pp. 583–596). Springer New York LLC. https://doi.org/10.1007/s10620-018-4939-1

Nishino, K., Nishida, A., Inoue, R., Kawada, Y., Ohno, M., Sakai, S., Inatomi, O., Bamba, S., Sugimoto, M., Kawahara, M., Naito, Y., & Andoh, A. (2018). Analysis of endoscopic brush samples identified mucosa-associated dysbiosis in inflammatory bowel disease. *Journal of Gastroenterology*, *53*(1), 95–106. https://doi.org/10.1007/s00535-017-1384-4

Noah, T. K., Donahue, B., & Shroyer, N. F. (2011). Intestinal development and differentiation. In *Experimental Cell Research* (Vol. 317, Issue 19, pp. 2702–2710). Academic Press Inc. https://doi.org/10.1016/j.yexcr.2011.09.006

Noah, T. K., Kazanjian, A., Whitsett, J., & Shroyer, N. F. (2010). SAM pointed domain ETS factor (SPDEF) regulates terminal differentiation and maturation of intestinal goblet cells. *Experimental Cell Research*, *316*(3), 452–465. https://doi.org/10.1016/j.yexcr.2009.09.020

Odenwald, M. A., & Turner, J. R. (2017). The intestinal epithelial barrier: A therapeutic target? In *Nature Reviews Gastroenterology and Hepatology* (Vol. 14, Issue 1, pp. 9–21). Nature Publishing Group. https://doi.org/10.1038/nrgastro.2016.169

Ong, M. L. D. M., Yeruva, S., Sailer, A., Nilsen, S. P., & Turner, J. R. (2020). Differential regulation of claudin-2 and claudin-15 expression in children and adults with malabsorptive disease. *Laboratory Investigation*, *100*(3), 483–490. https://doi.org/10.1038/s41374-019-0324-8

Oshima, T., Miwa, H., & Joh, T. (2008). Changes in the expression of claudins in active ulcerative colitis. *Journal of Gastroenterology and Hepatology (Australia)*, *23*(SUPPL. 2). https://doi.org/10.1111/j.1440-1746.2008.05405.x

Pabst, O., & Slack, E. (2020). IgA and the intestinal microbiota: the importance of being specific. In *Mucosal Immunology* (Vol. 13, Issue 1, pp. 12–21). Springer Nature. https://doi.org/10.1038/s41385-019-0227-4

Palm, N. W., De Zoete, M. R., Cullen, T. W., Barry, N. A., Stefanowski, J., Hao, L., Degnan, P. H., Hu, J., Peter, I., Zhang, W., Ruggiero, E., Cho, J. H., Goodman, A. L., & Flavell, R. A. (2014). Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell*, *158*(5), 1000–1010. https://doi.org/10.1016/j.cell.2014.08.006

Palmela, C., Chevarin, C., Xu, Z., Torres, J., Sevrin, G., Hirten, R., Barnich, N., Ng, S. C., & Colombel, J. F. (2018). Adherent-invasive Escherichia coli in inflammatory bowel disease. In *Gut* (Vol. 67, Issue 3, pp. 574–587). BMJ Publishing Group. https://doi.org/10.1136/gutjnl-2017-314903

Parekh, P. J., Balart, L. A., & Johnson, D. A. (2015). The influence of the gut microbiome on obesity, metabolic syndrome and gastrointestinal disease. In *Clinical and*

Translational Gastroenterology (Vol. 6, Issue 6). Nature Publishing Group. https://doi.org/10.1038/ctg.2015.16

Parviz, F., Matullo, C., Garrison, W. D., Savatski, L., Adamson, J. W., Ning, G., Kaestner, K. H., Rossi, J. M., Zaret, K. S., & Duncan, S. A. (2003). Hepatocyte nuclear factor 4α controls the development of a hepatic epithelium and liver morphogenesis. *Nature Genetics*, *34*(3), 292–296. https://doi.org/10.1038/ng1175

Peterson, L. W., & Artis, D. (2014). Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. In *Nature Reviews Immunology* (Vol. 14, Issue 3, pp. 141–153). https://doi.org/10.1038/nri3608

Pham, T. A. N., Clare, S., Goulding, D., Arasteh, J. M., Stares, M. D., Browne, H. P., Keane, J. A., Page, A. J., Kumasaka, N., Kane, L., Mottram, L., Harcourt, K., Hale, C., Arends, M. J., Gaffney, D. J., Dougan, G., & Lawley, T. D. (2014). Epithelial IL-22RA1-mediated fucosylation promotes intestinal colonization resistance to an opportunistic pathogen. *Cell Host and Microbe*, *16*(4), 504–516. https://doi.org/10.1016/j.chom.2014.08.017

Pickard, J. M., Maurice, C. F., Kinnebrew, M. A., Abt, M. C., Schenten, D., Golovkina, T. V., Bogatyrev, S. R., Ismagilov, R. F., Pamer, E. G., Turnbaugh, P. J., & Chervonsky, A. V. (2014). Rapid fucosylation of intestinal epithelium sustains host-commensal symbiosis in sickness. *Nature*, *514*(7524), 638–641. https://doi.org/10.1038/nature13823

Pine, G. M., Batugedara, H. M., & Nair, M. G. (2018). Here, there and everywhere: Resistin-like molecules in infection, inflammation, and metabolic disorders. In *Cytokine* (Vol. 110, pp. 442–451). Academic Press. https://doi.org/10.1016/j.cyto.2018.05.014

Podolsky, D. K., Gerken, G., Eyking, A., & Cario, E. (2009). Colitis-Associated Variant of TLR2 Causes Impaired Mucosal Repair Because of TFF3 Deficiency. *Gastroenterology*, *137*(1), 209–220. https://doi.org/10.1053/j.gastro.2009.03.007

Pope, J. L., Bhat, A. A., Sharma, A., Ahmad, R., Krishnan, M., Washington, M. K., Beauchamp, R. D., Singh, A. B., & Dhawan, P. (2014). Claudin-1 regulates intestinal epithelial homeostasis through the modulation of Notch-signalling. *Gut*, *63*(4), 622–634. https://doi.org/10.1136/gutjnl-2012-304241

Poritz, L. S., Harris, L. R., Kelly, A. A., & Koltun, W. A. (2011). Increase in the tight junction protein claudin-1 in intestinal inflammation. *Digestive Diseases and Sciences*, *56*(10), 2802–2809. https://doi.org/10.1007/s10620-011-1688-9

Pott, J., & Hornef, M. (2012). Innate immune signalling at the intestinal epithelium in homeostasis and disease. In *EMBO Reports* (Vol. 13, Issue 8, pp. 684–698). https://doi.org/10.1038/embor.2012.96

Prager, M., Buettner, J., & Buening, C. (2015). Genes involved in the regulation of intestinal permeability and their role in ulcerative colitis. *Journal of Digestive Diseases*, *16*(12), 713–722. https://doi.org/10.1111/1751-2980.12296

Prasad, S., Mingrino, R., Kaukinen, K., Hayes, K. L., Powell, R. M., MacDonald, T. T., & Collins, J. E. (2005). Inflammatory processes have differential effects on claudins 2, 3 and 4 in colonic epithelial cells. *Laboratory Investigation*, *85*(9), 1139–1162. https://doi.org/10.1038/labinvest.3700316

Priyamvada, S., Gomes, R., Gill, R. K., Saksena, S., Alrefai, W. A., & Dudeja, P. K. (2015). Mechanisms Underlying Dysregulation of Electrolyte Absorption in Inflammatory Bowel Disease-Associated Diarrhea. In *Inflammatory Bowel Diseases* (Vol. 21, Issue 12, pp. 2926–2935). Lippincott Williams and Wilkins.

https://doi.org/10.1097/MIB.0000000000000504

Propheter, D. C., Chara, A. L., Harris, T. A., Ruhn, K. A., & Hooper, L. V. (2017). Resistin-like molecule β is a bactericidal protein that promotes spatial segregation of the microbiota and the colonic epithelium. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(42), 11027–11033. https://doi.org/10.1073/pnas.1711395114

Prorok-Hamon, M., Friswell, M. K., Alswied, A., Roberts, C. L., Song, F., Flanagan, P. K., Knight, P., Codling, C., Marchesi, J. R., Winstanley, C., Hall, N., Rhodes, J. M., & Campbell, B. J. (2014). Colonic mucosa-associated diffusely adherent afaC+ Escherichia coli expressing lpfA and pks are increased in inflammatory bowel disease and colon cancer. *Gut*, *63*(5), 761–770. https://doi.org/10.1136/gutjnl-2013-304739

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D. R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., … Zoetendal, E. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59–65. https://doi.org/10.1038/nature08821

Qin, X., Caputo, F. J., Xu, D. Z., & Deitch, E. A. (2008). Hydrophobicity of mucosal surface and its relationship to gut barrier function. *Shock*, *29*(3), 372–376. https://doi.org/10.1097/SHK.0b013e3181453f4e

Quigley, E. M. M. (2011). Commensal bacteria: The link between IBS and IBD? In *Current Opinion in Clinical Nutrition and Metabolic Care* (Vol. 14, Issue 5, pp. 497–503). https://doi.org/10.1097/MCO.0b013e328348c033

Rabsch, W., Tschäpe, H., & Bäumler, A. J. (2001). Non-typhoidal salmonellosis: Emerging problems. In *Microbes and Infection* (Vol. 3, Issue 3, pp. 237–247). Elsevier Masson SAS. https://doi.org/10.1016/S1286-4579(01)01375-2

Radtke, F., & Clevers, H. (2005). Self-renewal and cancer of the gut: Two sides of a coin. In *Science* (Vol. 307, Issue 5717, pp. 1904–1909). https://doi.org/10.1126/science.1104815

Raju, P., Shashikanth, N., Tsai, P. Y., Pongkorpsakol, P., Chanez-Paredes, S., Steinhagen, P. R., Kuo, W. T., Singh, G., Tsukita, S., & Turner, J. R. (2020). Inactivation of paracellular cation-selective claudin-2 channels attenuates immune-mediated experimental colitis in mice. *Journal of Clinical Investigation*, *130*(10), 5197–5208. https://doi.org/10.1172/JCI138697

Rapozo, D. C. M., Bernardazzi, C., & De Souza, H. S. P. (2017). Diet and microbiota in inflammatory bowel disease: The gut in disharmony. In *World Journal of Gastroenterology* (Vol. 23, Issue 12, pp. 2124–2140). Baishideng Publishing Group Co., Limited. https://doi.org/10.3748/wjg.v23.i12.2124

Raupach, B., Peuschel, S. K., Monack, D. M., & Zychlinsky, A. (2006). Caspase-1-mediated activation of interleukin-1β (IL-1β) and IL-18 contributes to innate immune defenses against Salmonella enterica serovar typhimurium infection. *Infection and Immunity*, *74*(8), 4922–4926. https://doi.org/10.1128/IAI.00417-06

Rhee, K. J., Wu, S., Wu, X., Huso, D. L., Karim, B., Franco, A. A., Rabizadeh, S., Golub, J. E., Mathews, L. E., Shin, J., Balfour Sartor, R., Golenbock, D., Hamad, A. R., Gan, C. M., Housseau, F., & Sears, C. L. (2009). Induction of persistent colitis by a human commensal, enterotoxigenic Bacteroides fragilis, in wild-type C57BL/6 mice. *Infection and Immunity*, *77*(4), 1708–1718. https://doi.org/10.1128/IAI.00814-08

Rogier, E. W., Frantz, A. L., Bruno, M. E. C., & Kaetzel, C. S. (2014). Secretory IgA is

concentrated in the outer layer of colonic mucus along with gut bacteria. *Pathogens*, *3*(2), 390–403. https://doi.org/10.3390/pathogens3020390

Roodsant, T., Navis, M., Aknouch, I., Renes, I. B., van Elburg, R. M., Pajkrt, D., Wolthers, K. C., Schultsz, C., van der Ark, K. C. H., Sridhar, A., & Muncan, V. (2020). A Human 2D Primary Organoid-Derived Epithelial Monolayer Model to Study Host-Pathogen Interaction in the Small Intestine. *Frontiers in Cellular and Infection Microbiology*, *10*. https://doi.org/10.3389/fcimb.2020.00272

Rosenthal, R., Günzel, D., Krug, S. M., Schulzke, J. D., Fromm, M., & Yu, A. S. L. (2017). Claudin-2-mediated cation and water transport share a common pore. *Acta Physiologica*, *219*(2), 521–536. https://doi.org/10.1111/apha.12742

Rosenthal, Rita, Milatz, S., Krug, S. M., Oelrich, B., Schulzke, J. D., Amasheh, S., Günzel, D., & Fromm, M. (2010). Claudin-2, a component of the tight junction, forms a paracellular water channel. *Journal of Cell Science*, *123*(11), 1913–1921. https://doi.org/10.1242/jcs.060665

Rowan, F., Docherty, N. G., Murphy, M., Murphy, B., Coffey, J. C., & O'Connell, P. R. (2010). Desulfovibrio Bacterial Species Are Increased in Ulcerative Colitis. *Diseases of the Colon and Rectum*, *53*(11), 1530–1536. https://doi.org/10.1007/DCR.0b013e3181f1e620

Rutlin, M., Rastelli, D., Kuo, W. T., Estep, J. A., Louis, A., Riccomagno, M. M., Turner, J. R., & Rao, M. (2020). The Villin1 Gene Promoter Drives Cre Recombinase Expression in Extraintestinal Tissues. *CMGH*, *10*(4), 864-867.e5. https://doi.org/10.1016/j.jcmgh.2020.05.009

S. Ranhotra, H., L. Flannigan, K., Brave, M., Mukherjee, S., J. Lukin, D., A. Hirota, S., & Mani, S. (2016). Xenobiotic Receptor-Mediated Regulation of Intestinal Barrier Function and Innate Immunity. *Nuclear Receptor Research*, *3*. https://doi.org/10.11131/2016/101199

Samanta, P., Wang, Y., Fuladi, S., Zou, J., Li, Y., Shen, L., Weber, C., & Khalili-Araghi, F. (2018). Molecular determination of claudin-15 organization and channel selectivity. *Journal of General Physiology*, *150*(7), 949–968. https://doi.org/10.1085/jgp.201711868

Schirmer, M., Garner, A., Vlamakis, H., & Xavier, R. J. (2019). Microbial genes and pathways in inflammatory bowel disease. In *Nature Reviews Microbiology* (Vol. 17, Issue 8, pp. 497–511). Nature Publishing Group. https://doi.org/10.1038/s41579-019-0213-6

Schmitz, H., Barmeyer, C., Fromm, M., Runkel, N., Foss, H. D., Bentzel, C. J., Riecken, E. O., & Schulzke, J. D. (1999). Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. *Gastroenterology*, *116*(2), 301–309. https://doi.org/10.1016/S0016-5085(99)70126-5

Schneider, H., Pelaseyed, T., Svensson, F., & Johansson, M. E. V. (2018). Study of mucin turnover in the small intestine by in vivo labeling. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-24148-x

Schonhoff, S. E., Giel-Moloney, M., & Leiter, A. B. (2004). Minireview: Development and differentiation of gut endocrine cells. In *Endocrinology* (Vol. 145, Issue 6, pp. 2639–2644). https://doi.org/10.1210/en.2004-0051

Schultz, B. M., Salazar, G. A., Paduro, C. A., Pardo-Roa, C., Pizarro, D. P., Salazar-Echegarai, F. J., Torres, J., Riedel, C. A., Kalergis, A. M., álvarez-Lobos, M. M., & Bueno, S. M. (2018). Persistent Salmonella enterica serovar Typhimurium infection

increases the susceptibility of mice to develop intestinal inflammation. *Frontiers in Immunology*, *9*(MAY). https://doi.org/10.3389/fimmu.2018.01166

Schulzke, J. D., Bojarski, C., Zeissig, S., Heller, F., Gitter, A. H., & Fromm, M. (2006). Disrupted barrier function through epithelial cell apoptosis. *Annals of the New York Academy of Sciences*, *1072*, 288–299. https://doi.org/10.1196/annals.1326.027

Schütte, A., Ermund, A., Becker-Pauly, C., Johansson, M. E. V., Rodriguez-Pineiro, A. M., Bacḱhed, F., Mul̈ler, S., Lottaz, D., Bond, J. S., & Hansson, G. C. (2014). Microbial-induced meprin β cleavage in MUC2 mucin and a functional CFTR channel are required to release anchored small intestinal mucus. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(34), 12396–12401. https://doi.org/10.1073/pnas.1407597111

Schwartz, B., Algamas-Dimantav, A., Hertz, R., Natal, J., Kerman, A., Peri, I., & Bar-Tana, J. (2009). Inhibition of colorectal cancer by targeting hepatocyte nuclear factor-4α. *International Journal of Cancer*, *124*(5), 1081–1089. https://doi.org/10.1002/ijc.24041

Sekizuka, T., Ogasawara, Y., Ohkusa, T., & Kuroda, M. (2017). Characterization of Fusobacterium varium Fv113-g1 isolated from a patient with ulcerative colitis based on complete genome sequence and transcriptome analysis. *PLoS ONE*, *12*(12). https://doi.org/10.1371/journal.pone.0189319

Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biology*, *14*(8). https://doi.org/10.1371/journal.pbio.1002533

Shan, M., Gentile, M., Yeiser, J. R., Walland, A. C., Bornstein, V. U., Chen, K., He, B., Cassis, L., Bigas, A., Cols, M., Comerma, L., Huang, B., Blander, J. M., Xiong, H., Mayer, L., Berin, C., Augenlicht, L. H., Velcich, A., & Cerutti, A. (2013). Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals. *Science*, *342*(6157), 447–453. https://doi.org/10.1126/science.1237910

Shanahan, F. (2007). Irritable Bowel Syndrome: Shifting the Focus Toward the Gut Microbiota. In *Gastroenterology* (Vol. 133, Issue 1, pp. 340–342). W.B. Saunders. https://doi.org/10.1053/j.gastro.2007.05.030

Shen, L., Weber, C. R., Raleigh, D. R., Yu, D., & Turner, J. R. (2011). Tight junction pore and leak pathways: A dynamic duo. *Annual Review of Physiology*, *73*, 283–309. https://doi.org/10.1146/annurev-physiol-012110-142150

Shroyer, N. F., Helmrath, M. A., Wang, V. Y. C., Antalffy, B., Henning, S. J., & Zoghbi, H. Y. (2007). Intestine-Specific Ablation of Mouse atonal homolog 1 (Math1) Reveals a Role in Cellular Homeostasis. *Gastroenterology*, *132*(7), 2478–2488. https://doi.org/10.1053/j.gastro.2007.03.047

Shroyer, N. F., Wallis, D., Venken, K. J. T., Bellen, H. J., & Zoghbi, H. Y. (2005). Gfi1 functions downstream of Math1 to control intestinal secretory cell subtype allocation and differentiation. *Genes and Development*, *19*(20), 2412–2417. https://doi.org/10.1101/gad.1353905

Simpson, E. R. (2003). Sources of estrogen and their importance. *Journal of Steroid Biochemistry and Molecular Biology*, *86*(3–5), 225–230. https://doi.org/10.1016/S0960-0760(03)00360-1

Sladek, F. M., Zhong, W., Lai, E., & Darnell, J. E. (1990). Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. *Genes and Development*, *4*(12 B), 2353–2365. https://doi.org/10.1101/gad.4.12b.2353

Songhet, P., Barthel, M., Stecher, B., Müller, A. J., Kremer, M., Hansson, G. C., & Hardt,

W. D. (2011). Stromal IFN-γr-signaling modulates goblet cell function during salmonella Typhimurium infection. *PLoS ONE*, *6*(7). https://doi.org/10.1371/journal.pone.0022459

Specian, R. D., & Neutra, M. R. (1982). Regulation of intestinal goblet cell secretion. I. Role of parasympathetic stimulation. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *5*(4). https://doi.org/10.1152/ajpgi.1982.242.4.g370

Strugala, V., Dettmar, P. W., & Pearson, J. P. (2008). Thickness and continuity of the adherent colonic mucus barrier in active and quiescent ulcerative colitis and Crohn's disease. *International Journal of Clinical Practice*, *62*(5), 762–769. https://doi.org/10.1111/j.1742-1241.2007.01665.x

Su, D., Nie, Y., Zhu, A., Chen, Z., Wu, P., Zhang, L., Luo, M., Sun, Q., Cai, L., Lai, Y., Xiao, Z., Duan, Z., Zheng, S., Wu, G., Hu, R., Tsukamoto, H., Lugea, A., Liu, Z., Pandol, S. J., & Han, Y. P. (2016). Vitamin D signaling through induction of paneth cell defensins maintains gut microbiota and improves metabolic disorders and hepatic steatosis in animal models. *Frontiers in Physiology*, *7*(NOV). https://doi.org/10.3389/fphys.2016.00498

Su, L., Nalle, S. C., Shen, L., Turner, E. S., Singh, G., Breskin, L. A., Khramtsova, E. A., Khramtsova, G., Tsai, P. Y., Fu, Y. X., Abraham, C., & Turner, J. R. (2013). TNFR2 activates mlck-dependent tight junction dysregulation to cause apoptosis-mediated barrier loss and experimental colitis. *Gastroenterology*, *145*(2), 407–415. https://doi.org/10.1053/j.gastro.2013.04.011

Sun, J., Shen, X., Li, Y., Guo, Z., Zhu, W., Zuo, L., Zhao, J., Gu, L., Gong, J., & Li, J. (2016). Therapeutic potential to modify the mucus barrier in inflammatory bowel disease. In *Nutrients* (Vol. 8, Issue 1). MDPI AG. https://doi.org/10.3390/nu8010044

Suwandi, A., Galeev, A., Riedel, R., Sharma, S., Seeger, K., Sterzenbach, T., García Pastor, L., Boyle, E. C., Gal-Mor, O., Hensel, M., Casadesús, J., Baines, J. F., & Grassl, G. A. (2019). Std fimbriae-fucose interaction increases Salmonella-induced intestinal inflammation and prolongs colonization. *PLoS Pathogens*, *15*(7). https://doi.org/10.1371/journal.ppat.1007915

Suzuki, K., Meek, B., Doi, Y., Muramatsu, M., Chiba, T., Honjo, T., & Fagarasan, S. (2004). Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(7), 1981–1986. https://doi.org/10.1073/pnas.0307317101

Suzuki, T., Yoshinaga, N., & Tanabe, S. (2011). Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. *Journal of Biological Chemistry*, *286*(36), 31263–31271. https://doi.org/10.1074/jbc.M111.238147

Swidsinski, A., Loening-Baucke, V., Verstraelen, H., Osowska, S., & Doerffel, Y. (2008). Biostructure of Fecal Microbiota in Healthy Subjects and Patients With Chronic Idiopathic Diarrhea. *Gastroenterology*, *135*(2). https://doi.org/10.1053/j.gastro.2008.04.017

Tadesse, S., Corner, G., Dhima, E., Houston, M., Guha, C., Augenlicht, L., & Velcich, A. (2017). MUC2 mucin deficiency alters inflammatory and metabolic pathways in the mouse intestinal mucosa. *Oncotarget*, *8*(42), 71456–71470. https://doi.org/10.18632/oncotarget.16886

Takeichi, M. (2014). Dynamic contacts: Rearranging adherens junctions to drive epithelial remodelling. In *Nature Reviews Molecular Cell Biology* (Vol. 15, Issue 6, pp. 397–410). Nature Publishing Group. https://doi.org/10.1038/nrm3802

Tamura, A., Hayashi, H., Imasato, M., Yamazaki, Y., Hagiwara, A., Wada, M., Noda, T., Watanabe, M., Suzuki, Y., & Tsukita, S. (2011). Loss of claudin-15, but not claudin-2, causes Na+ deficiency and glucose malabsorption in mouse small intestine. *Gastroenterology*, *140*(3), 913–923. https://doi.org/10.1053/j.gastro.2010.08.006

Tanaka, H., Takechi, M., Kiyonari, H., Shioi, G., Tamura, A., & Tsukita, S. (2015). Intestinal deletion of claudin-7 enhances paracellular organic solute flux and initiates colonic inflammation in mice. *Gut*, *64*(10), 1529–1538. https://doi.org/10.1136/gutjnl-2014-308419

Taupin, D., & Podolsky, D. K. (2003). Trefoil factors: Initiators of mucosal healing. In *Nature Reviews Molecular Cell Biology* (Vol. 4, Issue 9, pp. 721–732). https://doi.org/10.1038/nrm1203

Teixeira, A., David, L., Reis, C. A., Costa, J., & Sobrinho-Simões, M. (2002). Expression of mucins (MUCI, MUC2, MUC5AC, and MUC6) and type I Lewis antigens in cases with and without Helicobacter pylori colonization in metaplastic glands of the human stomach. *Journal of Pathology*, *197*(1), 37–43. https://doi.org/10.1002/path.1083

Textoris, J., Ban, L. H., Capo, C., Raoult, D., Leone, M., & Mege, J. L. (2010). Sex-related differences in gene expression following Coxiella burnetii infection in mice: Potential role of circadian rhythm. *PLoS ONE*, *5*(8). https://doi.org/10.1371/journal.pone.0012190

Thomsson, K. A., Holmén-Larsson, J. M., Ångström, J., Johansson, M. E. V., Xia, L., & Hansson, G. C. (2012). Detailed O-glycomics of the Muc2 mucin from colon of wild-type, core 1- and core 3-transferase-deficient mice highlights differences compared with human MUC2. *Glycobiology*, *22*(8), 1128–1139. https://doi.org/10.1093/glycob/cws083

Tong, M., McHardy, I., Ruegger, P., Goudarzi, M., Kashyap, P. C., Haritunians, T., Li, X., Graeber, T. G., Schwager, E., Huttenhower, C., Fornace, A. J., Sonnenburg, J. L., McGovern, D. P. B., Borneman, J., & Braun, J. (2014). Reprograming of gut microbiome energy metabolism by the FUT2 Crohn's disease risk polymorphism. *ISME Journal*, *8*(11), 2193–2206. https://doi.org/10.1038/ismej.2014.64

Tripathi, M. K., Pratap, C. B., Dixit, V. K., Singh, T. B., Shukla, S. K., Jain, A. K., & Nath, G. (2016). Ulcerative colitis and its association with Salmonella species. *Interdisciplinary Perspectives on Infectious Diseases*, *2016*. https://doi.org/10.1155/2016/5854285

Turner, J. E., Stockinger, B., & Helmby, H. (2013). IL-22 Mediates Goblet Cell Hyperplasia and Worm Expulsion in Intestinal Helminth Infection. *PLoS Pathogens*, *9*(10). https://doi.org/10.1371/journal.ppat.1003698

Turner, J. R., Cohen, D. E., Mrsny, R. J., & Madara, J. L. (2000). Noninvasive in vivo analysis of human small intestinal paracellular absorption: Regulation by Na+-glucose cotransport. *Digestive Diseases and Sciences*, *45*(11), 2122–2126. https://doi.org/10.1023/A:1026682900586

Turner, Jerrold R. (2009). Intestinal mucosal barrier function in health and disease. *Nature Reviews Immunology*, *9*(11), 799–809. https://doi.org/10.1038/nri2653

Turner, Jerrold R., Rill, B. K., Carlson, S. L., Carnes, D., Kerner, R., Mrsny, R. J., & Madara, J. L. (1997). Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. *American Journal of Physiology - Cell Physiology*, *273*(4 42-4). https://doi.org/10.1152/ajpcell.1997.273.4.c1378

Ugolev, A. M., & De Laey, P. (1973). Membrane digestion a concept of enzymic hydrolysis

on cell membranes. In *BBA - Reviews on Biomembranes* (Vol. 300, Issue 2, pp. 105–128). https://doi.org/10.1016/0304-4157(73)90001-4

Uhlig, H. H., & Muise, A. M. (2017). Clinical Genomics in Inflammatory Bowel Disease. In *Trends in Genetics* (Vol. 33, Issue 9, pp. 629–641). Elsevier Ltd. https://doi.org/10.1016/j.tig.2017.06.008

Uniken Venema, W. T. C., Voskuil, M. D., Dijkstra, G., Weersma, R. K., & Festen, E. A. M. (2017). The genetic background of inflammatory bowel disease: from correlation to causality. In *Journal of Pathology* (Vol. 241, Issue 2, pp. 146–158). John Wiley and Sons Ltd. https://doi.org/10.1002/path.4817

Uronis, J. M., Mühlbauer, M., Herfarth, H. H., Rubinas, T. C., Jones, G. S., & Jobin, C. (2009). Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. *PLoS ONE*, *4*(6). https://doi.org/10.1371/journal.pone.0006026

Utech, M., Mennigen, R., & Bruewer, M. (2010). Endocytosis and recycling of tight junction proteins in inflammation. In *Journal of Biomedicine and Biotechnology* (Vol. 2010). https://doi.org/10.1155/2010/484987

Vaishnava, S., Behrendt, C. L., Ismail, A. S., Eckmann, L., & Hooper, L. V. (2008). Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(52), 20858–20863. https://doi.org/10.1073/pnas.0808723105

Vaishnava, S., Yamamoto, M., Severson, K. M., Ruhn, K. A., Yu, X., Koren, O., Ley, R., Wakeland, E. K., & Hooper, L. V. (2011). The antibacterial lectin RegIIIγ promotes the spatial segregation of microbiota and host in the intestine. *Science*, *334*(6053), 255–258. https://doi.org/10.1126/science.1209791

Van Der Flier, L. G., & Clevers, H. (2009). Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annual Review of Physiology*, *71*, 241–260. https://doi.org/10.1146/annurev.physiol.010908.163145

Van Der Heijden, M., & Vermeulen, L. (2019). Stem cells in homeostasis and cancer of the gut. In *Molecular Cancer* (Vol. 18, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12943-019-0962-x

Van Der Post, S., Jabbar, K. S., Birchenough, G., Arike, L., Akhtar, N., Sjovall, H., Johansson, M. E. V., & Hansson, G. C. (2019). Structural weakening of the colonic mucus barrier is an early event in ulcerative colitis pathogenesis. *Gut*, *68*(12), 2142–2151. https://doi.org/10.1136/gutjnl-2018-317571

Van der Sluis, M., De Koning, B. A. E., De Bruijn, A. C. J. M., Velcich, A., Meijerink, J. P. P., Van Goudoever, J. B., Büller, H. A., Dekker, J., Van Seuningen, I., Renes, I. B., & Einerhand, A. W. C. (2006). Muc2-Deficient Mice Spontaneously Develop Colitis, Indicating That MUC2 Is Critical for Colonic Protection. *Gastroenterology*, *131*(1), 117–129. https://doi.org/10.1053/j.gastro.2006.04.020

van Es, J. H., de Geest, N., van de Born, M., Clevers, H., & Hassan, B. A. (2010). Intestinal stem cells lacking the Math1 tumour suppressor are refractory to Notch inhibitors. *Nature Communications*, *1*, 18. https://doi.org/10.1038/ncomms1017

van Itallie, C. M., Holmes, J., Bridges, A., Gookin, J. L., Coccaro, M. R., Proctor, W., Colegio, O. R., & Anderson, J. M. (2008). The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. *Journal of Cell Science*, *121*(3), 298–305. https://doi.org/10.1242/jcs.021485

Van Itallie, C., Rahner, C., & Anderson, J. M. (2001). Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability.

Journal of Clinical Investigation, *107*(10), 1319–1327. https://doi.org/10.1172/JCI12464

Van Sommeren, S., Visschedijk, M. C., Festen, E. A. M., De Jong, D. J., Ponsioen, C. Y., Wijmenga, C., & Weersma, R. K. (2011). HNF4α and CDH1 are associated with ulcerative colitis in a Dutch cohort. *Inflammatory Bowel Diseases*, *17*(8), 1714–1718. https://doi.org/10.1002/ibd.21541

Vancamelbeke, M., Vanuytsel, T., Farré, R., Verstockt, S., Ferrante, M., Van Assche, G., Rutgeerts, P., Schuit, F., Vermeire, S., Arijs, I., & Cleynen, I. (2017). Genetic and Transcriptomic Bases of Intestinal Epithelial Barrier Dysfunction in Inflammatory Bowel Disease. *Inflammatory Bowel Diseases*, *23*(10), 1718–1729. https://doi.org/10.1097/MIB.0000000000001246

Vancamelbeke, M., & Vermeire, S. (2017). The intestinal barrier: a fundamental role in health and disease. In *Expert Review of Gastroenterology and Hepatology* (Vol. 11, Issue 9, pp. 821–834). Taylor and Francis Ltd. https://doi.org/10.1080/17474124.2017.1343143

VanDussen, K. L., & Samuelson, L. C. (2010). Mouse atonal homolog 1 directs intestinal progenitors to secretory cell rather than absorptive cell fate. *Developmental Biology*, *346*(2), 215–223. https://doi.org/10.1016/j.ydbio.2010.07.026

Vázquez-Martínez, E. R., García-Gómez, E., Camacho-Arroyo, I., & González-Pedrajo, B. (2018). Sexual dimorphism in bacterial infections. In *Biology of Sex Differences* (Vol. 9, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s13293-018-0187-5

Vazquez-Torres, A., Jones-Carson, J., Bäumler, A. J., Falkow, S., Valdivia, R., Brown, W., Le, M., Berggren, R., Parks, W. T., & Fang, F. C. (1999). Extraintestinal dissemination CD18-expressing phagocytes. *Nature*, *401*(October), 623–626.

Velcich, A., Yang, W. C., Heyer, J., Fragale, A., Nicholas, C., Viani, S., Kucherlapati, R., Lipkin, M., Yang, K., & Augenlicht, L. (2002). Colorectal cancer in mice genetically deficient in the mucin Muc2. *Science*, *295*(5560), 1726–1729. https://doi.org/10.1126/science.1069094

Verma, R., Verma, A. K., Ahuja, V., & Paul, J. (2010). Real-time analysis of mucosal flora in patients with inflammatory bowel disease in India. *Journal of Clinical Microbiology*, *48*(11), 4279–4282. https://doi.org/10.1128/JCM.01360-10

Visschedijk, M. C., Alberts, R., Mucha, S., Deelen, P., De Jong, D. J., Pierik, M., Spekhorst, L. M., Imhann, F., Van Der Meulen-de Jong, A. E., Van Janneke Der Woude, C., Van Bodegraven, A. A., Oldenburg, B., Löwenberg, M., Dijkstra, G., Ellinghaus, D., Schreiber, S., Wijmenga, C., Rivas, M. A., Franke, A., … Weersma, R. K. (2016). Pooled resequencing of 122 ulcerative colitis genes in a large Dutch cohort suggests population-Specific associations of rare variants in MUC2. *PLoS ONE*, *11*(8). https://doi.org/10.1371/journal.pone.0159609

Wada-Hiraike, O., Imamov, O., Hiraike, H., Hultenby, K., Schwend, T., Omoto, Y., Warner, M., & Gustafsson, J. Å. (2006). Role of estrogen receptor β in colonic epithelium. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(8), 2959–2964. https://doi.org/10.1073/pnas.0511271103

Walker, C. R., Hautefort, I., Dalton, J. E., Overweg, K., Egan, C. E., Bongaerts, R. J., Newton, D. J., Cruickshank, S. M., Andrew, E. M., & Carding, S. R. (2013). Intestinal intraepithelial lymphocyte-enterocyte crosstalk regulates production of bactericidal angiogenin 4 by paneth cells upon microbial challenge. *PLoS ONE*, *8*(12). https://doi.org/10.1371/journal.pone.0084553

Wang, L., Llorente, C., Hartmann, P., Yang, A. M., Chen, P., & Schnabl, B. (2015). Methods to determine intestinal permeability and bacterial translocation during liver disease. In *Journal of Immunological Methods* (Vol. 421, pp. 44–53). Elsevier B.V. https://doi.org/10.1016/j.jim.2014.12.015

Wang, M. L., Shin, M. E., Knight, P. A., Artis, D., Silberg, D. G., Suh, E., & Wu, G. D. (2005). Regulation of RELM/FIZZ isoform expression by Cdx2 in response to innate and adaptive immune stimulation in the intestine. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *288*(5 51-5). https://doi.org/10.1152/ajpgi.00442.2004

Wang, Y. Y., Liu, X. L., & Zhao, R. (2019). Induction of pyroptosis and its implications in cancer management. In *Frontiers in Oncology* (Vol. 9, Issue SEP). Frontiers Media S.A. https://doi.org/10.3389/fonc.2019.00971

Weber, C. R., Liang, G. H., Wang, Y., Das, S., Shen, L., Yu, A. S. L., Nelson, D. J., & Turner, J. R. (2015). Claudin-2-dependent paracellular channels are dynamically gated. *ELife*, *4*(NOVEMBER2015). https://doi.org/10.7554/eLife.09906

Weber, C. R., Nalle, S. C., Tretiakova, M., Rubin, D. T., & Turner, J. R. (2008). Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and may contribute to early neoplastic transformation. *Laboratory Investigation*, *88*(10), 1110–1120. https://doi.org/10.1038/labinvest.2008.78

Weber, C. R., Raleigh, D. R., Su, L., Shen, L., Sullivan, E. A., Wang, Y., & Turner, J. R. (2010). Epithelial myosin light chain kinase activation induces mucosal interleukin-13 expression to alter tight junction ion selectivity. *Journal of Biological Chemistry*, *285*(16), 12037–12046. https://doi.org/10.1074/jbc.M109.064808

Weersma, R. K., Stokkers, P. C. F., Cleynen, I., Wolfkamp, S. C. S., Henckaerts, L., Schreiber, S., Dijkstra, G., Franke, A., Nolte, I. M., Rutgeerts, P., Wijmenga, C., & Vermeire, S. (2009). Confirmation of multiple Crohn's disease susceptibility loci in a large Dutch-Belgian cohort. *American Journal of Gastroenterology*, *104*(3), 630–638. https://doi.org/10.1038/ajg.2008.112

Wlodarska, M., Luo, C., Kolde, R., d'Hennezel, E., Annand, J. W., Heim, C. E., Krastel, P., Schmitt, E. K., Omar, A. S., Creasey, E. A., Garner, A. L., Mohammadi, S., O'Connell, D. J., Abubucker, S., Arthur, T. D., Franzosa, E. A., Huttenhower, C., Murphy, L. O., Haiser, H. J., … Xavier, R. J. (2017). Indoleacrylic Acid Produced by Commensal Peptostreptococcus Species Suppresses Inflammation. *Cell Host and Microbe*, *22*(1), 25-37.e6. https://doi.org/10.1016/j.chom.2017.06.007

Woodfint, R. M., Chen, P. R., Ahn, J., Suh, Y., Hwang, S., Lee, S. S., & Lee, K. (2017). Identification of the MUC2 promoter as a strong promoter for intestinal gene expression through generation of transgenic quail expressing GFP in gut epithelial cells. *International Journal of Molecular Sciences*, *18*(1). https://doi.org/10.3390/ijms18010196

Woting, A., & Blaut, M. (2018). Small intestinal permeability and gut-transit time determined with low and high molecular weight fluorescein isothiocyanate-dextrans in C3H mice. *Nutrients*, *10*(6). https://doi.org/10.3390/nu10060685

Yamashita, M. S. de A., & Melo, E. O. (2018). Mucin 2 (MUC2) promoter characterization: an overview. In *Cell and Tissue Research* (Vol. 374, Issue 3, pp. 455–463). Springer Verlag. https://doi.org/10.1007/s00441-018-2916-9

Yang, Q., Bermingham, N. A., Finegold, M. J., & Zoghbi, H. Y. (2001). Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. *Science*,

294(5549), 2155–2158. https://doi.org/10.1126/science.1065718

Ye, D., & Ma, T. Y. (2008). Cellular and molecular mechanisms that mediate basal and tumour necrosis factor-α-induced regulation of myosin light chain kinase gene activity. *Journal of Cellular and Molecular Medicine*, *12*(4), 1331–1346. https://doi.org/10.1111/j.1582-4934.2008.00302.x

Yilmaz, B., Juillerat, P., Øyås, O., Ramon, C., Bravo, F. D., Franc, Y., Fournier, N., Michetti, P., Mueller, C., Geuking, M., Pittet, V. E. H., Maillard, M. H., Rogler, G., Abdelrahman, K., Ademi, G., Aepli, P., Thomas, A., Anderegg, C., Antonino, A. T., … Macpherson, A. J. (2019). Microbial network disturbances in relapsing refractory Crohn's disease. *Nature Medicine*, *25*(2), 323–336. https://doi.org/10.1038/s41591-018-0308-z

Yu, K., Lujan, R., Marmorstein, A., Gabriel, S., & Hartzell, H. C. (2010). Bestrophin-2 mediates bicarbonate transport by goblet cells in mouse colon. *Journal of Clinical Investigation*, *120*(5), 1722–1735. https://doi.org/10.1172/JCI41129

Zamani, S., Hesam Shariati, S., Zali, M. R., Asadzadeh Aghdaei, H., Sarabi Asiabar, A., Bokaie, S., Nomanpour, B., Sechi, L. A., & Feizabadi, M. M. (2017). Detection of enterotoxigenic Bacteroides fragilis in patients with ulcerative colitis. *Gut Pathogens*, *9*(1). https://doi.org/10.1186/s13099-017-0202-0

Zarepour, M., Bhullar, K., Montero, M., Ma, C., Huang, T., Velcich, A., Xia, L., & Vallance, B. A. (2013). The mucin muc2 limits pathogen burdens and epithelial barrier dysfunction during salmonella enterica serovar typhimurium colitis. *Infection and Immunity*, *81*(10), 3672–3683. https://doi.org/10.1128/IAI.00854-13

Zeissig, S., Bojarski, C., Buergel, N., Mankertz, J., Zeitz, M., Fromm, M., & Schulzke, J. D. (2004). Downregulation of epithelial apoptosis and barrier repair in active Crohn's disease by tumour necrosis factor α antibody treatment. *Gut*, *53*(9), 1295–1302. https://doi.org/10.1136/gut.2003.036632

Zeissig, S., Bürgel, N., Günzel, D., Richter, J., Mankertz, J., Wahnschaffe, U., Kroesen, A. J., Zeitz, M., Fromm, M., & Schulzke, J. D. (2007). Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. *Gut*, *56*(1), 61–72. https://doi.org/10.1136/gut.2006.094375

Zha, L., Garrett, S., & Sun, J. (2019). Salmonella Infection in Chronic Inflammation and Gastrointestinal Cancer. *Diseases*, *7*(1), 28. https://doi.org/10.3390/diseases7010028

Zhang, M., & Wu, C. (2020). The relationship between intestinal goblet cells and the immune response. In *Bioscience Reports* (Vol. 40, Issue 10). Portland Press Ltd. https://doi.org/10.1042/BSR20201471

Zheng, H., Pritchard, D. M., Yang, X., Bennett, E., Liu, G., Liu, C., & Ai, W. (2009). KLF4 gene expression is inhibited by the notch signaling pathway that controls goblet cell differentiation in mouse gastrointestinal tract. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *296*(3). https://doi.org/10.1152/ajpgi.90393.2008

Zhong, Z. A., Sun, W., Chen, H., Zhang, H., Lay, Y. A. E., Lane, N. E., & Yao, W. (2015). Optimizing tamoxifen-inducible Cre/loxp system to reduce tamoxifen effect on bone turnover in long bones of young mice. *Bone*, *81*, 614–619. https://doi.org/10.1016/j.bone.2015.07.034

Zhu, M. J., Sun, X., & Du, M. (2018). AMPK in regulation of apical junctions and barrier function of intestinal epithelium. In *Tissue Barriers* (Vol. 6, Issue 2, pp. 1–13). Taylor and Francis Inc. https://doi.org/10.1080/21688370.2018.1487249

Zuo, L., Kuo, W. T., & Turner, J. R. (2020). Tight Junctions as Targets and Effectors of Mucosal Immune Homeostasis. In *CMGH* (Vol. 10, Issue 2, pp. 327–340). Elsevier Inc. https://doi.org/10.1016/j.jcmgh.2020.04.001

Zwiers, A., Fuss, I. J., Leijen, S., Mulder, C. J., Kraal, G., & Bouma, G. (2008). Increased expression of the tight junction molecule claudin-18 A1 in both experimental colitis and ulcerative colitis. *Inflammatory Bowel Diseases*, *14*(12), 1652–1659. https://doi.org/10.1002/ibd.20695

9. ANNEXES

Supplemental introduction

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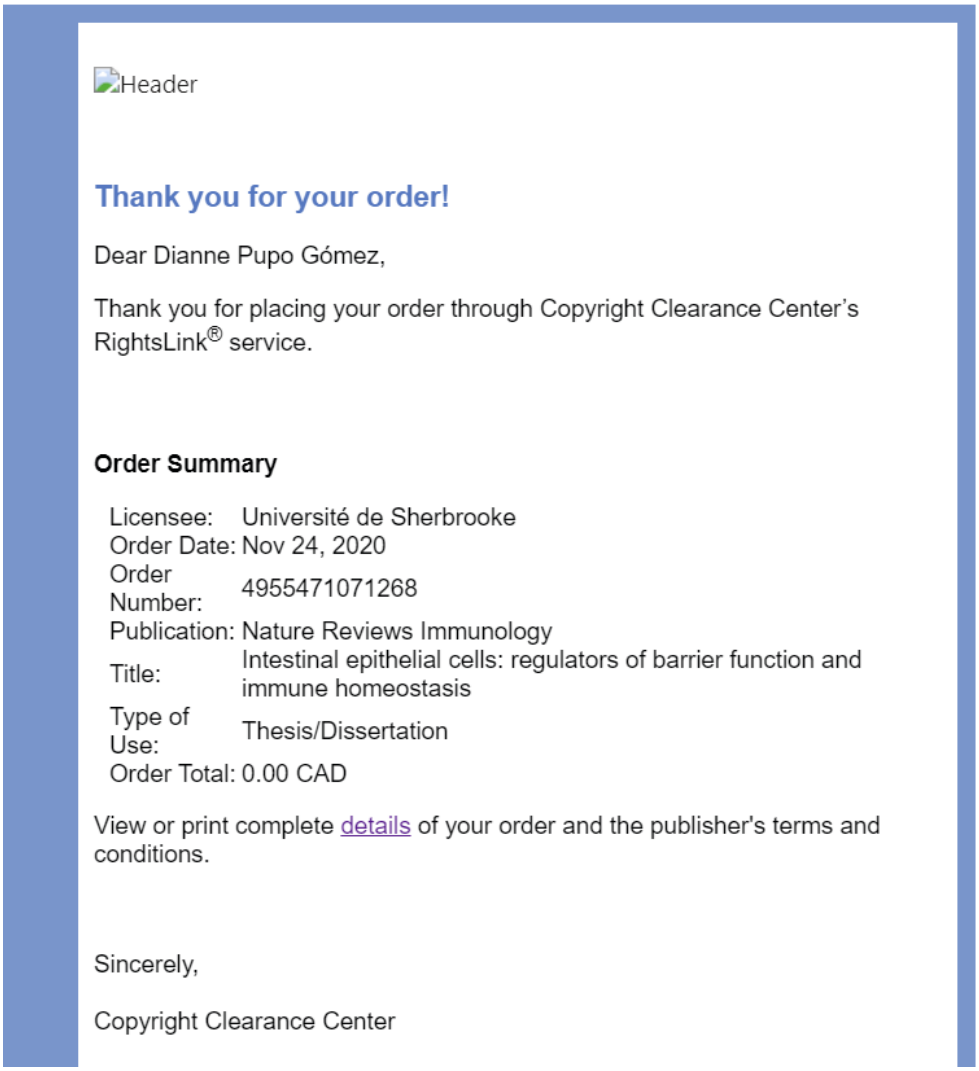

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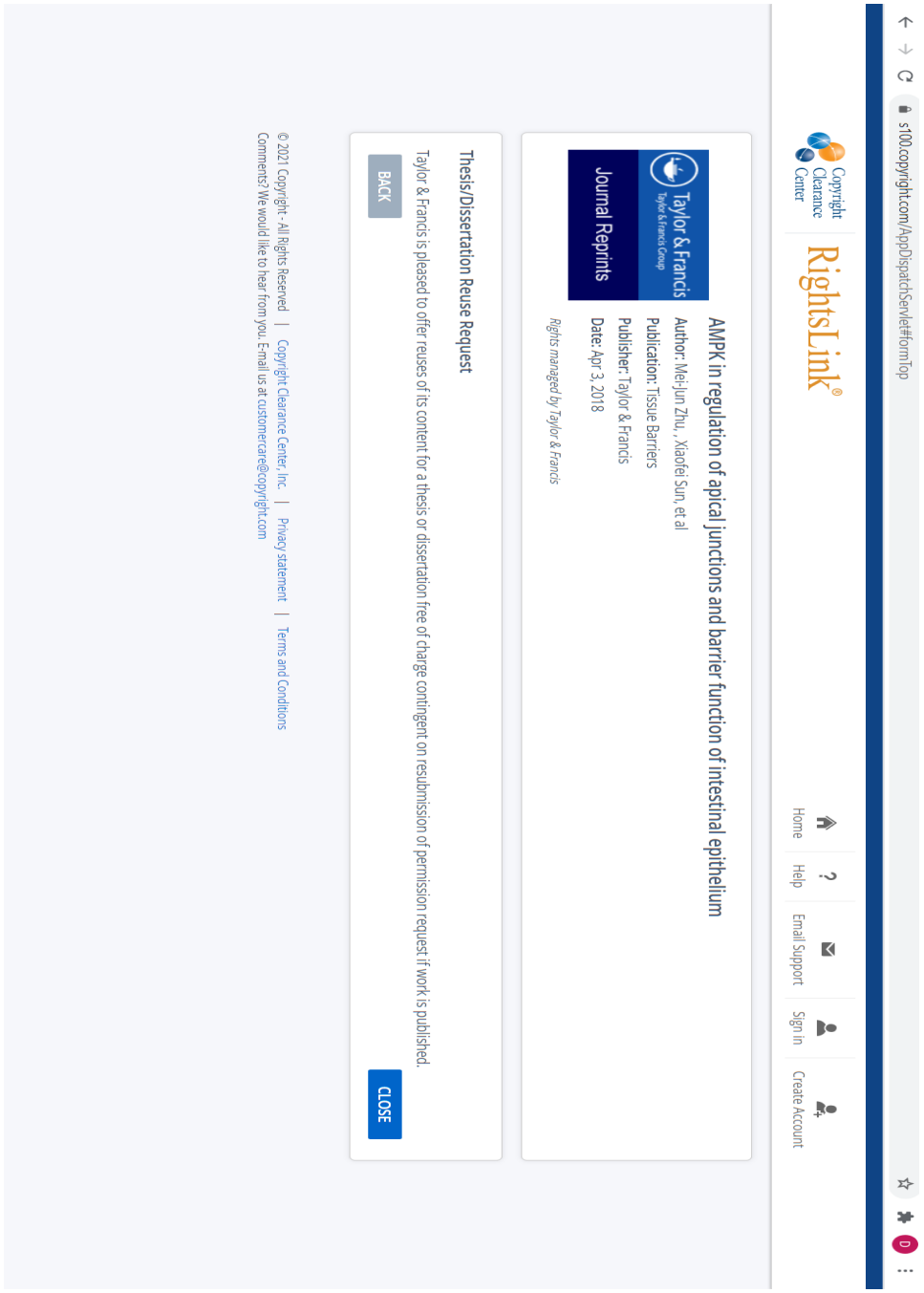

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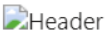

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Supplemental materials and methods

3D organoid culture from mouse small intestine and colon

Before starting manipulations of the murine tissues, the semi-viscous growth medium Matrigel (BD, Corning # 354230) and different compounds of the conditional medium (N2, B27, N-acetylcysteine (Nac), EGF) were placed to thaw on ice (see table 1 and 2).

The jejunum or colon of one *Hnf4a*$^{\Delta IEC\text{-}ind}$ mouse was harvested, opened longitudinally and washed several times in cold 1x PBS. Subsequently, the tissue was cut into small fragments of approximately 1.5 cm, recovered in a 50 ml Falcon tube and repeatedly and vigorously shaken in 1x PBS eliminating unwanted debris such as feces.

Jejunum:

The jejunum fragments were transferred into a sterile Falcon tube and incubated for 5 min on ice in a 30 mM EDTA solution pH 8 that was diluted in 1x PBS (20 ml). This solution was then replaced with a same volume of fresh solution and further incubated 20 min on ice. Next, the EDTA solution was removed and 40 ml of 1x PBS was added to the tube and shook vigorously for 5 min in order to obtain a good number of dissociated crypts in the solution.

Colon:

The colon fragments were placed in a Falcon tube containing 5mM EDTA pH 8, diluted in 1x PBS for 1 h on ice with shaking. After removing the EDTA, the segments were collected by gravity sedimentation and transferred to a Petri dish with 10 ml of sterile 1x PBS. Then, the epithelium was gently scraped until the tissue was slightly translucent. Next, the crypts contained in the Petri dish were collected in a sterile Falcon tube, and then 10 ml of 1x PBS was again added to the plate in order to recover all the tissue. Subsequently, the tissue was broken down by vigorous pipetting (x6 up and down).

Both colon and jejunum cells were filtered through a 70 µm cell strainer. The cells were centrifuged at 1500 g for 5 min and 3 successive washes of the pellet were carried out in ad-DF^{+++} medium in sterile condition. Next, the cells were collected by centrifugation at 1500 g for 5 min and resuspended in 1 ml of Matrigel, applying 20 µl/well of this medium in a 48-well plate. The plate was quickly incubated for 15 min at 37ºC after which 250 µl/well of

WENR medium with reagent Y27632 (ROCK inhibitor, 10 µM) was added. Finally, the conditional medium was changed every 3 days in the absence of Y27632 until the passage of the organoids.

Table 1: Composition of the ad-FD^{+++} medium

ad-FD^{+++}

Reactive	**Volume**
Advanced-DMEM/F12	500 ml
Glutamax (L-glutamine (2.5 mM)	5 ml
HEPES	5 ml
Penicillin (100 U/ml) / Streptomycin (0.1 mg/ml) antibiotic	5 ml

Table 2: Composition of the WENR medium

Conditional medium WENR

Reactive	**Volum (20 ml)**	**Catalog. number**	**Provider**
Ad-DF^{+++}	6 ml	12634-010	Life technologies
n-Acetylcysteine 500 mM	500 µl	A9165	Sigma
EGF (50 µg/ml)	20 µl	PMG8045	Life technologies
Conditional medium Noggin (10%)	2 ml		
Conditional medium R-spondin1 (10%)	2 ml		
B27 supplement	400 µl	17504-044	Gibco
N2 supplement	200 µl	17502-048	Gibco
Conditional medium Wnt3A (50%, colon)	10 ml		

Passage of a 3D organoid culture

For the organoids passage, all pipettes were previously coated with ad-DF^{+++} medium containing 10% of FBS. First, the medium was aspirated from the culture wells containing the Matrigel dome with the organoids and 1 ml / 4 wells of cold ad-DF^{+++} medium was added. The domes were then mechanically broken using a pipette tip and the content of 8 wells was combined into a 15 ml Falcon tube containing 3 ml of cold ad-DF^{+++}. To break the Matrigel and the organoids, 10-12 pipetting up and down were carried out and the organoid suspension was deposited to a new 50 ml Falcon tube with the same volume of ad-AF^{+++} medium to

solubilize the Matrigel. The suspension was centrifuged at 1500 g for 5 min at 4ºC and the cell pellet was resuspended in a new volume of Matrigel according to the desired passage split (1: 1 or 1: 2). 20 µl/well of the resuspended cells were distributed in a 48-well plate. The organoids were rapidly incubated at 37ºC for 15 min and 250 µl of WENR medium with reagent Y27632 was added to each well. The plate was incubated at 37ºC and the growth medium devoid of reagent Y27632 was changed every 3 days.

2D Monolayer from a 3D organoid culture

The monolayers were grown on Transwell membranes of 0.4 µm pore size and previously coated with Matrigel. For each Transwell membrane, 250 µl of a 1:30 dilution of Matrigel in ad-FD^{+++} medium was covered and incubated for 2 h at 37ºC. Subsequently, the solution was stirred and 400 µl/membrane of WENR medium with reagent Y27632 without NAC was added while incubating the plate with the inserts at 37ºC. Next, was aspirated the culture medium containing the Matrigel domes with the jejunal or colon organoids and added 1 ml/4 wells of ad-FD^{+++} medium. The domes were then mechanically broken using a pipette tip and also disrupted by vigorously pipetting up and down (6 times). The organoid suspension from 8 wells was collected and added to a 50 ml Falcon tube containing the same volume of 0.25% trypsin-EDTA (4 ml) and incubated for 30 min on ice. This was followed by a second incubation step at 37ºC for 10 min. 3 volumes of ad-FD^{+++} (24 ml) was then added in order to inactivate the trypsin. The cell suspension was centrifuged at 1500 g for 5 min, the pellet resuspended in 2 ml of ad-FD^{+++} containing 10% FBS and the organoids were disrupted by pipetting up and down (10 times). Individual cells were then counted in order to distribute 1.5–2.0 x10^{6} cells per Transwell. Next, the suspension was centrifuged at 1500 g x 5 min, and the organoids were resuspended in an eppendorf with 1 ml of 1x PBS. A new centrifugation step was performed at 20,000 g x 30 seconds and a volume of WENR medium with reagent Y27632 and without NAC equivalent to 100 µl/well was added to the pellet, depending on the number of membranes to be evaluated. After homogenizing the cell suspension, 100 µl were added in the wells previously coated with 400 µl of medium. In addition, 1 ml of medium was added in the outer chamber of the transwell membrane. The plate was gently shaken and incubated at 37ºC for two weeks, changing the growth medium

every 2 days (WENR medium without Y27632 and NAC). These steps were repeated depending on the number of cells to be used and the design of the experiment.

Supplemental results

Table 3: Statistical analysis of the CFU obtained in mutant and control mice feces for each day of infection. The bacterial load for the days 2, 3 and 4 (D2-D4) pi of each group of mice was evaluated in comparison to the D1 of the experiment. A non-parametric *Mann-Whitney* test was performed.

	D1 Ctl vs Dn Ctl (male)	D1 Mut vs Dn Mut (male)	D1 Ctl vs Dn Ctl (female)	D1 Mut vs Dn Mut (male)
D2	*** p=0.0002 (550-fold)	*** p=0.0006 (77-fold)	** p=0.0022 (154-fold)	** p=0.0079 (263-fold)
D3	** p=0.0070 (30-fold)	** p=0.0011 (43-fold)	** p=0.0022 (36-fold)	* p=0.0159 (128-fold)
D4	ns p=0.1866 (4.4-fold)	ns p=0.1049 (3.3-fold)	* p=0.0152 (7.3-fold)	ns p=0.1429 (17-fold)

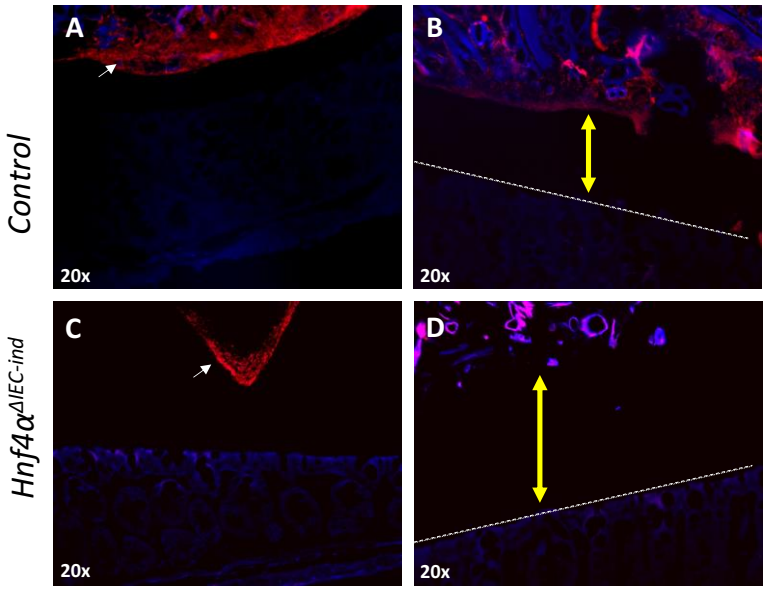

Fig. 1: *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice have a thick internal mucus layer. Bacteria were localized using the general bacterial probe EUB338-Cy3(red) in Carnoy's fixed colon sections. Nuclei were counterstained with DAPI (blue). Bacteria were mainly located in the outer mucus layer (white arrows) of both control (A) and *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice (C). A continuous and thick inner mucus layer was observed in Hnf4α$^{\Delta IEC\text{-}ind}$ mice (D) when compared to control mice (B) (double arrows yellow). Magnification 20x, (n=3)

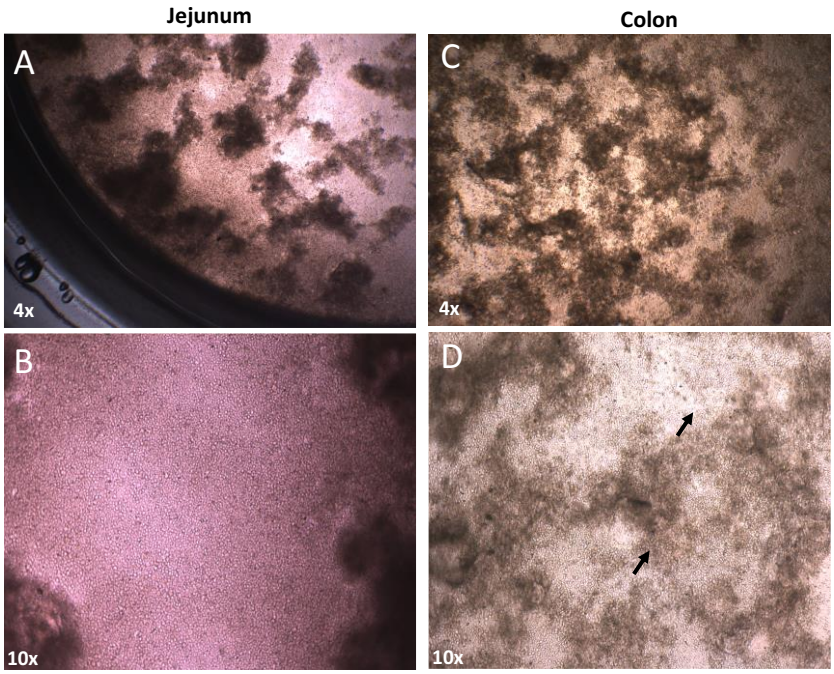

Fig. 2: Epithelial 2D-Monolayers established from intestinal organoids derived from *Hnf4a*$^{\Delta IEC\text{-}ind}$ male mice. Organoids-derived crypts were seeded on top of thick dome of Matrigel-coated polystyrene plates. After growth, organoids were disrupted and single cells were seeded on a thin layer of Matrigel/medium-coated Transwell membrane. A) Representative bright field images of jejunum monolayers after 15 days of culture at magnification 4x (A) and 10x (B). (n=3). Representative bright field images of colon monolayers after 7 days of culture at magnification 4x (C) and 10x (D). Black arrows point to holes in the monolayer.